

# Changes in the concentration of particular hormones and carbohydrates in apple shoots after “bending” respectively chemical treatments and relationship to the flower induction process

**Nopporn Boonplod**

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*Für meine liebe Frau*

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## TABLE OF CONTENTS

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## TABLE OF CONTENTS

	Page
<b>1 Introduction</b> .....	1
<b>2 Review of literature</b> .....	2
2.1 Alternate bearing .....	2
2.2 Flower induction .....	2
2.2.1 Flower induction hypothesis .....	2
2.2.2 Flower induction in apple trees .....	3
<b>3 Materials and methods</b> .....	5
3.1 Plant materials .....	5
3.2 Treatments .....	6
3.2.1 Shoot bending .....	6
3.2.2 Spraying with Alar plus Ethrel .....	6
3.3 Hormone analysis .....	7
3.3.1 Hormone extraction .....	7
3.3.2Purification .....	8
3.3.3 Quantification of hormones .....	10
3.4 Sugar and starch analysis .....	10
3.5 Weather data .....	12
<b>4.Results</b> .....	14

## TABLE OF CONTENTS

---

4.1 Experiments in 2001 .....	14
4.1.1 Effect of bending on hormonal changes in apical portion of apple tree shoots .....	14
4.1.2 Effect of bending on hormonal changes in shoot and leave of apple seedling cv. Golden Delicious .....	23
4.1.3 Effect of Alar plus Ethrel treatment on hormonal changes in growing and non growing shoot of apple trees .....	26
4.2 Experiments in 2002 .....	35
4.2.1 Effect of bending on hormonal changes in apical portion of apple tree shoots .....	35
4.2.2 Effect of Alar plus Ethrel treatment on hormonal changes in growing shoot of apple trees .....	42
4.3 Experiment in 2003 .....	49
4.3.1 Effect of Alar plus Ethrel treatment on changes in hormones, starch and sugar contents in apical portion of apple tree shoots .....	49
<b>5 Discussion .....</b>	<b>66</b>
<b>6 Conclusion and outlook .....</b>	<b>74</b>
<b>7 Summary .....</b>	<b>75</b>
<b>8 Zusammenfassung .....</b>	<b>77</b>
<b>9 References .....</b>	<b>80</b>

**List of abbreviations**

ABA	: Absciscic acid
CKs	: Cytokinins
CV	Cultivar
CONC.	Concentration
DW	: Dry weight
IAA	: Indole-3-acetic acid
i-Ade	: Isopentenyl-adenine
i-Ado	: Isopentenyl-adenosine
FI	: Flower induction
g	Gram
GAs	: Gibberellins
GA <sub>3</sub>	: Gibberellic acid
M	: Molar (Mol l <sup>-1</sup> )
MH	Maleic hydrazide
min	Minute
ml	Mililiter
mm	Milimeter
ng	Nanogram
PVP	: Polyvinylpyrrolidone
rpm	: Round per minute
TIBA	2,3,5-triiodobenzoic acid
Z/ZR	: Zeatin/Zeatin riboside

## 1 INTRODUCTION

Apples are grown throughout the temperate climatic zones. The major producing countries are France Italy and Germany for Europe, USA for the Americas and China and Turkey for the Asian region (O'ROURKE, 2003). In 2003, the total production was approximately 57,938,065 metric tons with 20,609,650 metric tons China contributes most to the world production followed by the USA, France, Poland, Italy, Russia and Germany (FAO 2003). The primary market for most apples produced around the world is for domestic fresh consumption. Where processing facilities are not available, apples that are not marketable as fresh fruit are used for livestock feed or are wasted. In a few countries, such as the USA, Germany and Australia, a large market for processed apple products such as slices, pie fillings, dried apples, applesauce, juice or cider has been developed. Specific cultivars were, and continue to be, grown because of their suitability for these processed products (O'ROURKE, 2003). However, apple production has been declining in many growing areas. One of the most important causes is alternate bearing behavior, i.e. the apple trees flower profusely in one year followed by a year of less or no flowering, resulting in an inconsistent yield return from the orchard. However, the alternate bearing behavior depends on cultivars. 'Boskoop', 'Elstar' and 'Idared' for example show stronger alternate bearing behavior than 'Golden Delicious' (SILBEREISEN *et al.*, 1996). The main flower induction (FI) period occurs the year before flowering in early summer, but it can extend into early autumn under some conditions. Apple flowers can be initiated in both terminal and axillary buds of shoots and terminal buds of spurs (DENNIS, 2003). It was reported that fruits containing many seeds or too vigorously growing shoots cause alternate bearing, which should be counterbalanced by conventional cultural practices as well as by the application of bioregulators (BUBAN, 2003). The temporary restriction of the competition between vigorous shoot growth and the potential flower buds is an efficient procedure in regulating FI (JONES, *et al.*, 1989). Therefore the conventional cultural practices, such as bending shoots and chemical treatments have been widely used to regulate FI in apple trees. The physiological mode of action of these methods in FI is still unknown. However, phytohormones have been proved to be involved as important messengers in this process of regulation (BUBAN, 2003) or may act as stimulative signals (METZGER, 1995). In this experiment, changes in endogenous hormones, starch and sugar contents were investigated after bending treatments and spraying apple trees with Alar and Ethrel for improving FI. It is hoped that the results of these investigations may yield some solutions in unraveling the cause of the alternate bearing behavior in apple trees.



## 2 REVIEW OF LITERATURE

### 2.1 Alternate bearing

Alternate bearing has been investigated in apples longer and more extensively than in any other fruit tree species. This phenomenon has encountered to be a problem of woody fruit trees in many countries (WILLIAM and EDGERTON, 1974). Over yield and vigorous growing shoots have been identified as the inhibiting effects on FI of apple trees (BUBAN and FAUST, 1982). A heavy fruit load in one year is reflected in a strong reduction of flower production and fruit yield for the following season resulting in ‘on’ and ‘off’ years with respect to fruit load (TROMP, 2000). ABBOTT (1970) indicated that shoot growth must be ceased before FI. However, a later study provided no proof that shoot growth has to stop before FI begins (FAUST, 1989). Gibberellins and auxin have been investigated as the inhibiting signal transport from seeds and apical buds of growing shoots to spurs and lateral buds, the sites of FI (BANGERTH, 1997; CALLEJAS and BANGERTH, 1997). The reduction of carbohydrate and nitrogenous reserves in roots of trees with a heavy crop load, has also been put forward as a cause of reduced flowering and, therefore, directly linked to alternate bearing (GOLDSCHMIDT and GOLOMB, 1982). Reducing vegetative growth and strong sinks, such as fruit thinning, in the overbearing season can enhance return flowering (MCARTNEY *et al.*, 1995).

### 2.2 Flower induction

#### 2.2.1 Flower induction hypothesis

##### *Florigen:*

The florigen concept was based on the transmissibility of substances or signals across grafts between a “donor” shoot and vegetative “recipients”. It was proposed of that the florigen, a flower-promoting hormone, gets produced in mature leaves under favorable conditions and is transported via phloem to a competent meristem (reviewed in LANG, 1952; EVANS, 1971). On the other hand, flowering promoting signals originating in roots are presumably transmitted in the xylem with the transpiration stream to a shoot meristem

(BERNIER *et al.*, 1993). The possible existence of an “antiflorigen”, a flower-inhibiting hormone, was also reported. Over the years many researchers tried to identify both substances in the phloem sap, but so far their chemical nature has remained elusive.

*Assimilate diversion hypothesis:*

At the core of the nutrient diversion hypothesis, as it pertains to the relationship between vegetative and reproductive development, is a notion that a critical part of the shoot apical meristem is relatively deprived of nutrients during reproductive development or must receive a higher level of assimilates for gene expression than required for vegetative development. The class of chemicals, climatic conditions and management that mobilize nutrients at shoot apical meristematic tissues or suppress competitive sinks for assimilates at times appropriate for floral initiation assume greater importance in the nutrient diversion hypothesis. Thus auxin, cytokinins and gibberellins should increase assimilate transport towards the kind of treated tissues and may promote or inhibit flowering depending upon the tissues treated and their specificity of action (SACHS, 1977).

*Multifactorial control:*

This hypothesis postulates that several chemicals, assimilates and known phytohormones participate in floral induction as promoters or inhibitors (BERNIER *et al.*, 1981; BERNIER, 1988). Of all the currently known plant hormones, gibberellins (GAs) are most strongly associated with flowering (PHARIS and KING, 1985). GA treatments have been shown to inhibit flower bud induction and possibly differentiation in many fruit species (GOLDSCHMIDT *et al.*, 1997). Cytokinins on the other hand have been reported to promote flowering in many fruit trees, e.g. apple, litchi and longan (SKOGERBØ, 1992; STERN *et al.*, 2003; CHEN, 1997).

### 2.2.2 FI in apple trees

FI in annual/biennial plants refers to the change from vegetative to the reproductive phase, which is not relevant for grafted fruit trees. Main FI in trees occurs in early summer, but can extend into early autumn under some conditions. Most of the flower parts are already present by early autumn (visible microscopically) and continue to develop in temperate climate until low temperature prevents further growth. In most temperate fruit-growing areas buds become dormant in late summer/early autumn and winter chilling is

necessary to permit renewed growth the following year. Apple flowers can be initiated in both terminal and axillary buds of shoots and terminal buds of spurs. The flower buds are mixed, containing both vegetative and reproductive parts (DENNIS, 2003). The concurrence of fruit containing seeds and too vigorous vegetative growth causes inhibition of FI (BUBÁN, 2003). The temporary restriction of the competition between vigorous shoot growth and the potential flower buds is an efficient means in regulating flower initiation (WILLIAMS, 1973, cit. in JONES et al., 1989). Horticultural treatments such as shoot bending and application of growth inhibitors reduce growth and favor flowering (LUCKWILL and SILVA, 1979, SANYAL and BANGERTH, 1998). It is unknown what is the physiological mode of action of these methods regarding FI, but it has been proven that phytohormones are important messengers in the process of this regulation. As the site of the sources of inhibition (fruits with seeds and shoot apices) are remote to the site of flower initiation, one has to postulate the necessity of transferring the message. It is still uncertain, whether the signal arrives immediately from the growing seeds and shoot tips to the site of its action or through other organ, e.g. leaves (BANGERTH, 1997). There are still open questions concerning the signal function of phytohormones in this context.

### 3 MATERIALS AND METHODS

#### 3.1 Plant materials

Apple trees (*Malus domestica* Borkh.), ‘Golden Delicious’, ‘Boskoop’, ‘Elstar’ and ‘Idared’ (all with M9 rootstock) all located at the experimental station of the Department of Special Crop Cultivation and Crop Physiology, University of Hohenheim, were used in these experiments. The ‘Golden Delicious’ cultivar was used additionally in seedling experiments. The experiments were carried out at the end of May to the end of June 2001-2003. Vegetative growth was visible at the beginning of May. The vegetative growth consisted of bourse shoots and elongated shoots, which could be distinguished in to growing and non-growing shoots. ‘Boskoop’, ‘Elstar’ and ‘Idared’ are irregular bearing, whereas ‘Golden Delicious’ is regarded as regular bearing cultivars. The other differences among the cultivars are shown in table 3.1.

Table 3.1 Cultivar characters

	Golden D.	Boskoop	Elstar	Idared
Alternate behavior	weak	strong	strong	middle to weak
Branch vigour development	middle	strong	strong	middle
Growth of whole tree	middle	very strong	middle to strong	middle to weak
Ploidy	diploid	triploid	diploid	diploid

## 3.2 Treatments

### 3.2.1 Bending

#### 3.2.1.1 Experiment in 2001

Vertical elongated shoots of ‘Golden Delicious’, ‘Boskoop’ and ‘Elstar’ apple trees were bent downward and horizontal elongated shoots upward on 29<sup>th</sup> May (this method was adopted from SANYAL and BANGERTH, 1998). Terminal parts of shoots including the apical meristem and were collected at 2, 4, 8, 12, 16 and 25 days after bending. In seedling experiments the vertical terminal stems were bent downward on 29<sup>th</sup> of October. Terminal parts of shoots were collected at 2, 4, 6, 9 and 12 days after bending. Terminal parts were used because this is the meristem competent to be transformed into a flower bud.

#### 3.2.1.2 Experiment in 2002

Vertical elongated shoots of ‘Golden Delicious’ and ‘Idared’ apple trees were bent downward and horizontal elongated shoots upward on 26<sup>th</sup> of May. Terminal parts of bending treatments and controls were collected at 1, 3, 4, 5, 7, 9 and 11 days after bending. ‘Idared’ replaced Boskoop and ‘Elstar’ in this year because the latter cvs. were not available in 2002.

The terminal parts of shoots were immediately frozen in liquid nitrogen and kept at –20 °C. The samples were then freeze-dried and stored at –20 °C until extraction.

### 3.2.2 Spraying

#### 3.2.2.1 Experiment in 2001

‘Golden Delicious’ and ‘Boskoop’ apple trees were sprayed with 1000 ppm Alar plus 250 ppm Ethrel on 14<sup>th</sup> of June. Growing and non-growing shoots were collected at 1, 5, 8 and 10 days after spraying.

### 3.2.2.2 Experiment in 2002

‘Golden Delicious’ and ‘Idared’ apple trees were sprayed with 1000 ppm Alar plus 250 ppm Ethrel on 28<sup>th</sup> of May. Growing shoots were collected at 1, 2, 6, 8 and 10 days after spraying. Wood and bark separated by peeling with a knife and were collected at 7, 8, 9 and 12 days after spraying.

### 3.2.2.3 Experiment in 2003

‘Golden Delicious’ and ‘Elstar’ apple trees were sprayed with 1000 ppm Alar plus 250 ppm Ethrel on 19<sup>th</sup> of June. Terminal parts of growing and non-growing shoots were collected at 1, 4, 6 and 11 days after spraying.

The plant samples were immediately frozen in liquid nitrogen and kept at –20 °C. The frozen samples were then freeze-dried and stored at –20 °C until extraction. For IAA leaf diffusates, excised shoots tips were placed with their stem ends into the cavities of multititer culture plates. Each cavity was filled with 2.5 ml of 0.1 M phosphate buffer, pH 6.2 and incubated under 100% RH in darkness at 20°C for 20 hours. Thereafter the shoots were removed and the plates frozen and kept at –20°C until analysis.

## 3.3 Hormones analysis

### 3.3.1 Hormone extraction

Terminal parts of shoots (approximately 400 mg dry weight [DW]) were homogenized in 40 ml of 80% cold methanol. The plant extracts were kept in darkness at 4°C overnight. The extracts were then filtrated through G4-glasssinter-filters (max. pore size 10-16 µm). After that the extracts were reduced to almost complete dryness in a rotary evaporator under vacuum at 40°C under low pressure and dissolved 3 times with 4 ml each of 0.01 M ammonium acetate, pH 7.5, by using an ultrasonic bath. The extracts were pooled (approx. 12 ml) and subsequently frozen at –20°C overnight. After thawing, the extracts were centrifuged at 22,000 rpm at 4°C for 25 min.

### 3.3.2. Purification

#### 3.3.2.1 Plant samples (see Figure 3.1 for column assembly and purification)

The centrifuged supernatant was passed through a combination of preconditioned columns, filled with 10 ml polyvinylpyrrolidone suspension (PVP; Sigma Chemical Co., Deisenhofen, Germany), and 4 ml DEAE-Sephadex A-25 (Pharmacia, Freiburg, Germany) followed by a C<sub>18</sub> Sep-Pak cartridge for cytokinins (Waters, Dreieich, Germany), arranged as described in Figure 3.1. (modified from BERTLING and BANGERTH, 1995). Pre-conditioning of the C<sub>18</sub> Sep-Pak cartridges:

- 2 x washing with 4 ml 100% methanol in 0.1 M acetic acid
- 2 x washing with 4 ml 0.1 M acetic acid

The column combination was eluted with 3x10 ml 0.01 M ammonium acetate (pH 7.5). At this point, the acidic hormones (free IAA, ABA and GAs) were bound as anions to the DEAE Sephadex and the cytokinins got trapped in the Sep-Pak cartridge. The Sep-Pak cartridge for cytokinins was then replaced by preconditioned cartridges for ABA and GAs. After the PVP column, which retained phenolic compounds and other impurities, discarded the ABA and GAs were eluted from the Sephadex into the Sep-Pak cartridge, with 15 ml 0.75 M acetic acid. Finally, the preconditioned Sep-Pak cartridge for IAA was attached and IAA was eluted with 10 ml 2.0 M acetic acid.

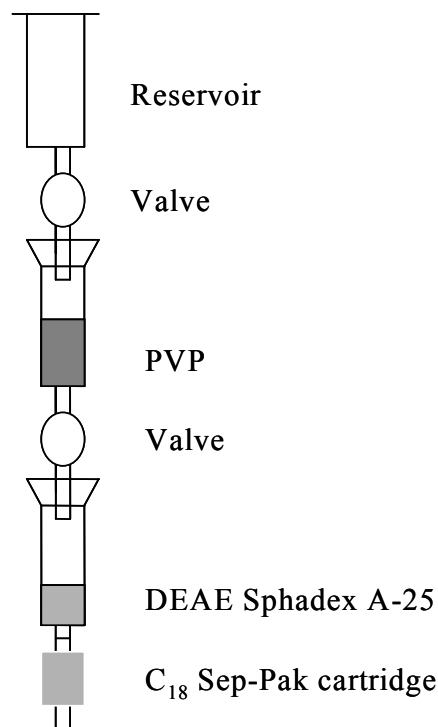


Figure 3.1. The column system used for hormone purification

After removing the Sep-Pak cartridges from the column, they were washed with 4 ml 0.1 M acetic acid. To elute the bound hormones from the Sep-Pak cartridges, 4 ml of the following solutions were used:

- 30 % methanol in 0.1 M acetic acid for Z/ZR
- 40% methanol in 0.1 M acetic acid for IAA
- 65% methanol in 0.1 M acetic acid for ABA and GAs
- 80% methanol in 0.1 M acetic acid for iAde/iAdo

The extracted hormones were pipetted into small vials in triplicates and evaporated overnight in a vacuum concentrator. Zeatin/zeatin riboside (Z/ZR), *N*<sup>6</sup>-( $\Delta^2$ - isopentenyl) adenine/ *N*<sup>6</sup>-( $\Delta^2$ -isopentenyl) adenosine (iAde/iAdo), indole-3-acetic acid (IAA), gibberellins (GA<sub>1,3,20</sub>) and abscisic acid (ABA) were determined by radio-immunoassay (RIA) as described under 3.3.3.



### 3.3.3.2 IAA leaf diffusates

The buffer solution of four cavities were pooled, adjusted to pH 3 with acetic acid and passed through a C<sub>18</sub> Sep-Pak cartridge (Waters, Eschborn, Germany). After that the cartridge was washed with 4 ml 0.1 M acetic acid and the IAA eluted with 4 ml 40% of methanol in 0.1 M acetic acid. Aliquots of this eluate were pipetted into small vials in triplicates and evaporated overnight in a vacuum concentrator. RIA was used to determine the IAA concentration (BOHNER and BANGERTH, 1988).

### 3.3.3 Quantification of hormones

Hormones were quantified by radio-immunoassay with polyclonal antibodies. Fractions containing free IAA, ABA and GA<sub>s</sub> were methylated with diazomethane prior to analysis. Antibodies used were raised against free IAA, ABA, GA<sub>1</sub> Z/ZR and iAde/i-Ado (for details see BOHNER and BANGERTH 1988). Cross-reactions of the GA<sub>3</sub> antibody used were determined by BERTLING and BANGERTH (1995), to be about 90% with GA<sub>1</sub> and GA<sub>20</sub>. Therefore, the GA determined by means of this antibody are expressed as GA<sub>3</sub> equivalents and called GAs in the following text.

Sample preparation and ethylene determination (Method see SANYAL and BANGERTH, 1998)

## 3.4 Sugar and starch analysis

### 3.4.1 Chemicals

*Anthrone reagent:* This was prepared as described by TREVELYAN and HARRISON (1952) by dissolving 0.2 g of Anthrone (97%) in 100 ml of H<sub>2</sub>SO<sub>4</sub> solution, made by adding 500 ml of concentrated acid to 200 ml of deion. H<sub>2</sub>O. The reagent was allowed to stand for 30-40 min. with occasional shaking until it was perfectly clear. The reagent was freshly prepared each day and used within 12 hours.

*PAHBAH:* This was prepared by dissolving 0.5 g p-hydroxybenzoic acid (PAHBAH) in 100 ml of alkaline solution, made by taking up 14.7 g Tri-sodiumcitrate hydrate, 1.47 g CaCl<sub>2</sub> and 20 g NaOH in a small volume of deion. H<sub>2</sub>O. First Tri-sodiumcitrate hydrate

and  $\text{CaCl}_2$  solution were mixed and then NaOH solution was added and filled up with deion.  $\text{H}_2\text{O}$  to a volume of 1 liter.

*Invertase:* Prepared by dissolving 12.5 mg Invertase in 50 ml of deion.  $\text{H}_2\text{O}$

### 3.4.2 Standards

*Starch:*

Three replications: 10/20/30/40/50/60 mg Starch in 100 ml deion.  $\text{H}_2\text{O}$

*Glucose:*

25 to 700 mg glucose were dissolved in 100 ml deion.  $\text{H}_2\text{O}$

*Sucrose:*

25 to 400 mg sucrose in 100 ml deion.  $\text{H}_2\text{O}$

### 3.4.3 Plant extracts

One hundred mg of plant samples were weighted (DW) and placed in a test tube. Five 5 ml deion.  $\text{H}_2\text{O}$  were added into the test tube and kept for 1 h. with occasional shaking. After that extracts were centrifuged at 4000 rpm at 20 °C for 30 min. The supernatants were separated from pellets and used for reducing sugar and sucrose analysis, while the pellets were used for starch analysis.

### 3.4.4 Reducing sugar and sucrose analysis

One ml of supernatant was diluted with 4 ml deion.  $\text{H}_2\text{O}$ . The dilution was aliquoted to 100  $\mu\text{l}$  into a cylinder glass tube (150 X 25 mm). Adding 0.4 ml of deion.  $\text{H}_2\text{O}$  for reducing sugar analysis and 0.4 ml of invertase for sucrose analysis. After 30 min., 5 ml of PAHBAH was added in each tube, and subsequently boiled at 100 °C for 4 min. Finally 10 ml of deion.  $\text{H}_2\text{O}$ . was added to each tube and the concentrations of reducing sugar and sucrose were determined by spectrophotometer at a wavelength of 415 nm.

### 3.4.5 Starch analysis

The pellet of the plant samples was dissolved with 6 ml deion. H<sub>2</sub>O and boiled at 100°C for 20 min. After cooling down 0.5 ml of acetate buffer and 0.3 ml of  $\alpha$ -amylase solution to the samples were added at 20°C incubated for 20 min then boiled at 100°C for 10 min. The volume of the sample solution was filled to 25 ml with deion. H<sub>2</sub>O. One ml aliquots were pipetted into cylinder glass tubes, which contained 5 ml of anthrone and placed on ice. Triplicates were performed with each plant sample. The solution was homogenized during cooling on ice. After that the glass cylinders with the samples were boiled at 100 °C for 10 min and then cooled down in cold water for 5 min. The resulting glucose concentrations were determined by spectrophotometer at a wavelength of 600 nm.

### 3.5 Weather data

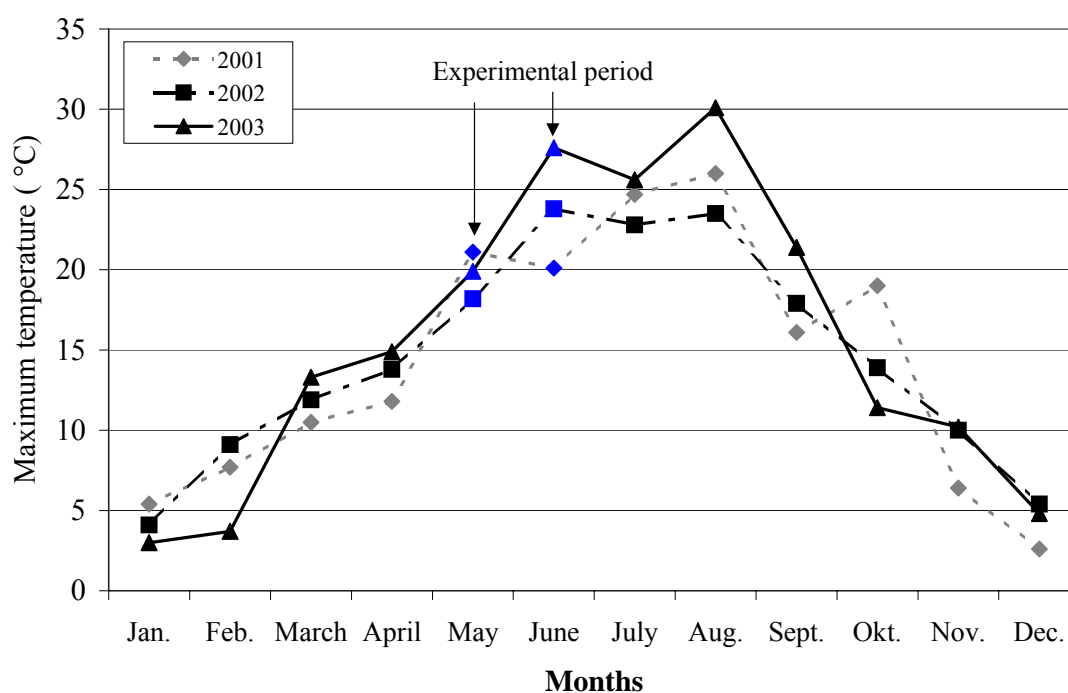


Figure 3.2 Temperature changes in the experimental station of the University of Hohenheim, 2001-2003.

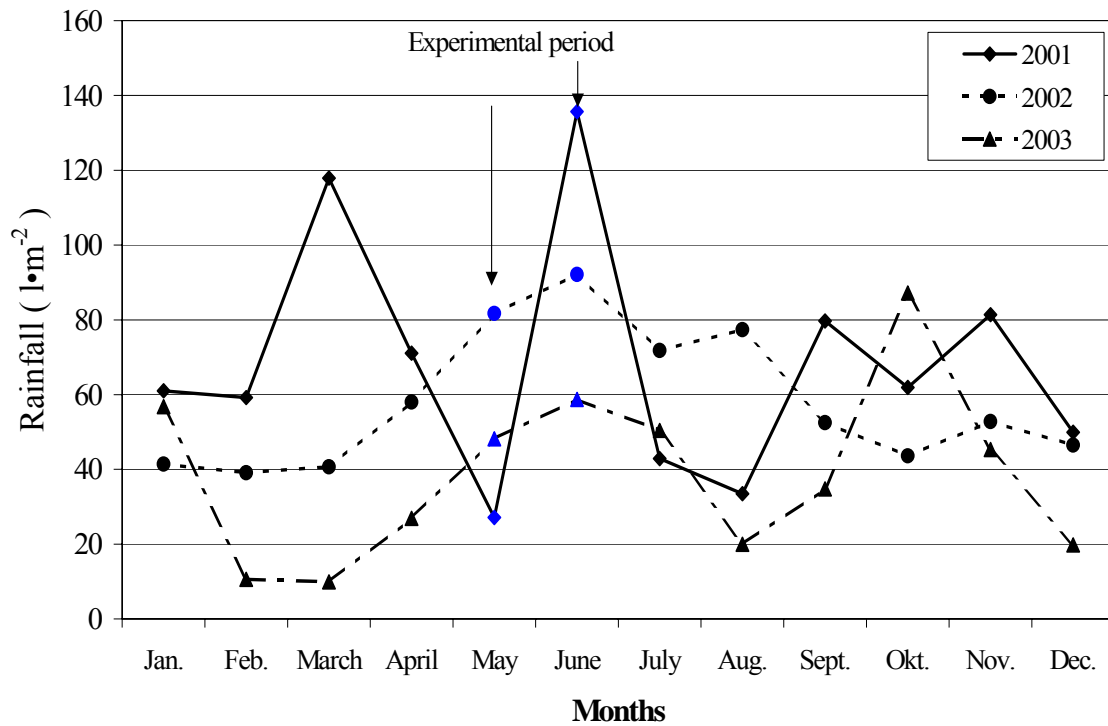


Figure 3.3 Rain fall in the experimental station of the University of Hohenheim, 2001-2003.

Statistic: due to the rather laborious procedure of hormonal analyses and the great number of samples replicate measurements could not be performed in the available time, except for ethylene, which can be rapidly determined.

## 4 RESULTS

### 4.1 Experiments in 2001

#### 4.1.1 Effect of bending on hormonal changes in apical portions of apple tree shoots

##### 4.1.1.1 Cytokinins

With one exception, Z/ZR concentrations in the apical portion of the shoot of cv. Golden Delicious increased already two days after the start of the treatment when compared to control (Figure 4.1). Contrary to that in cvs. Boskoop and Elstar Z/ZR concentrations in bended down material was similar or even lower than in the control. Bending up treatments also seemed to increase free Z/ZR in cv. Boskoop, while there were no differences among treatments in cv. Elstar (Figure 4.2, 4.3). Furthermore, iAde/iAdo concentrations were erratic and showed no clear overall tendencies in all three cvs. (Figure 4.4, 4.5 and 4.6).

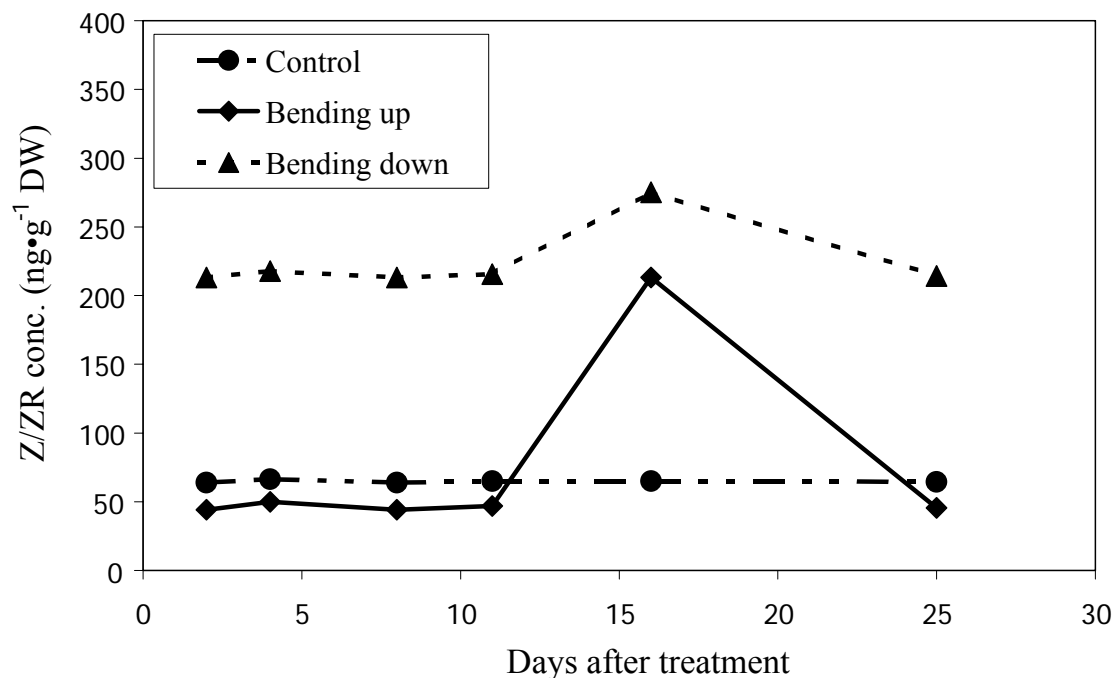


Figure 4.1. Z/ZR concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

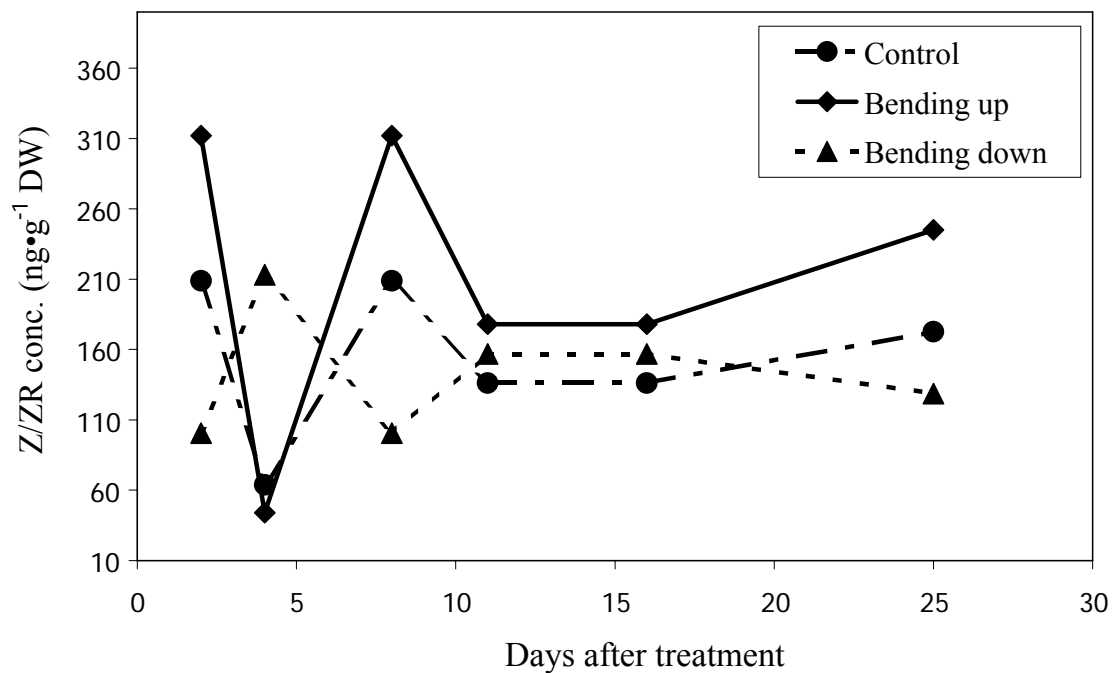


Figure 4.2. Z/ZR concentrations in the apical portion of the shoot of cv. Boskoop after bending treatments

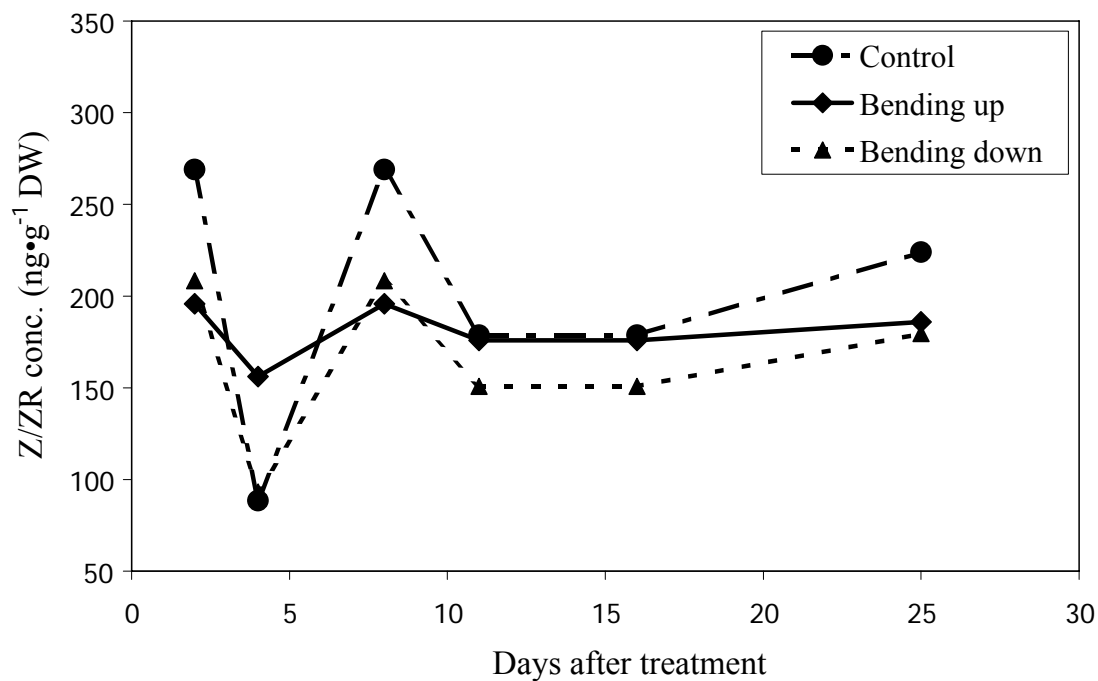


Figure 4.3. Z/ZR concentrations in the apical portion of the shoot of cv. Elstar after bending treatments

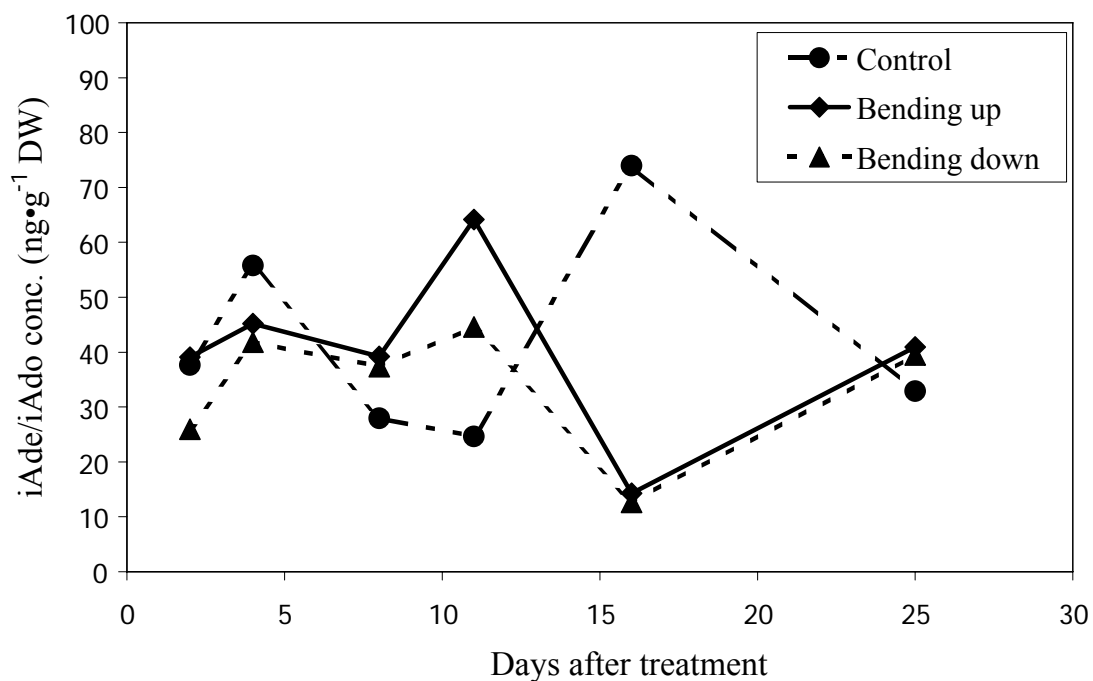


Figure 4.4. iAde/iAdo concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

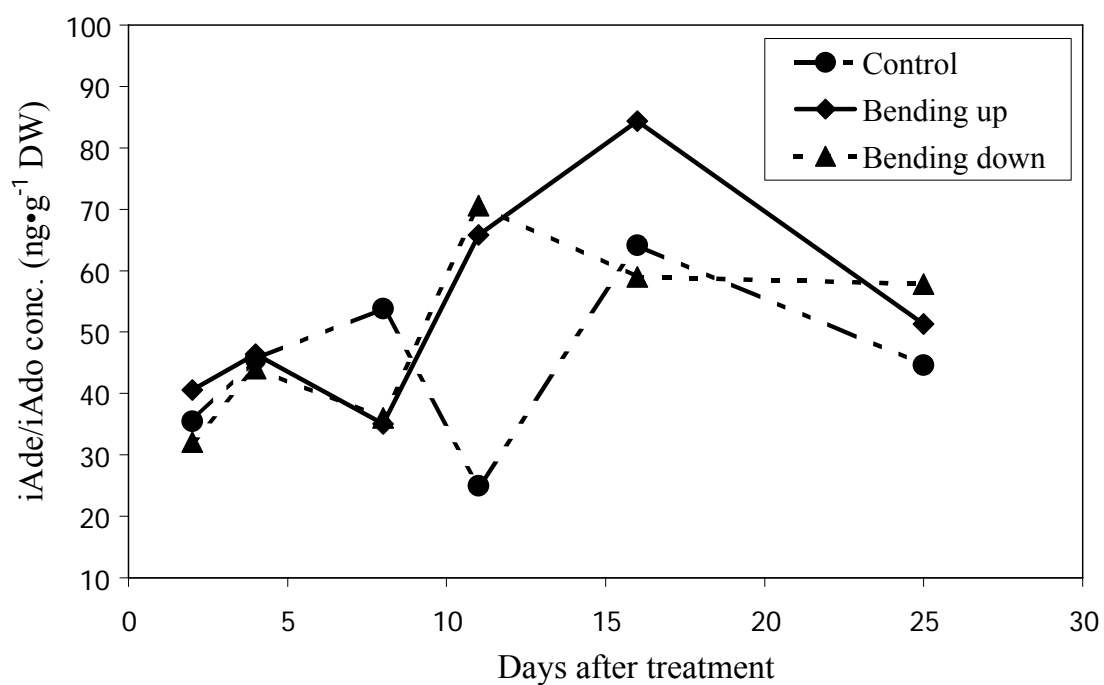


Figure 4.5. iAde/iAdo concentrations in the apical portion of the shoot of cv. Boskoop after bending treatments

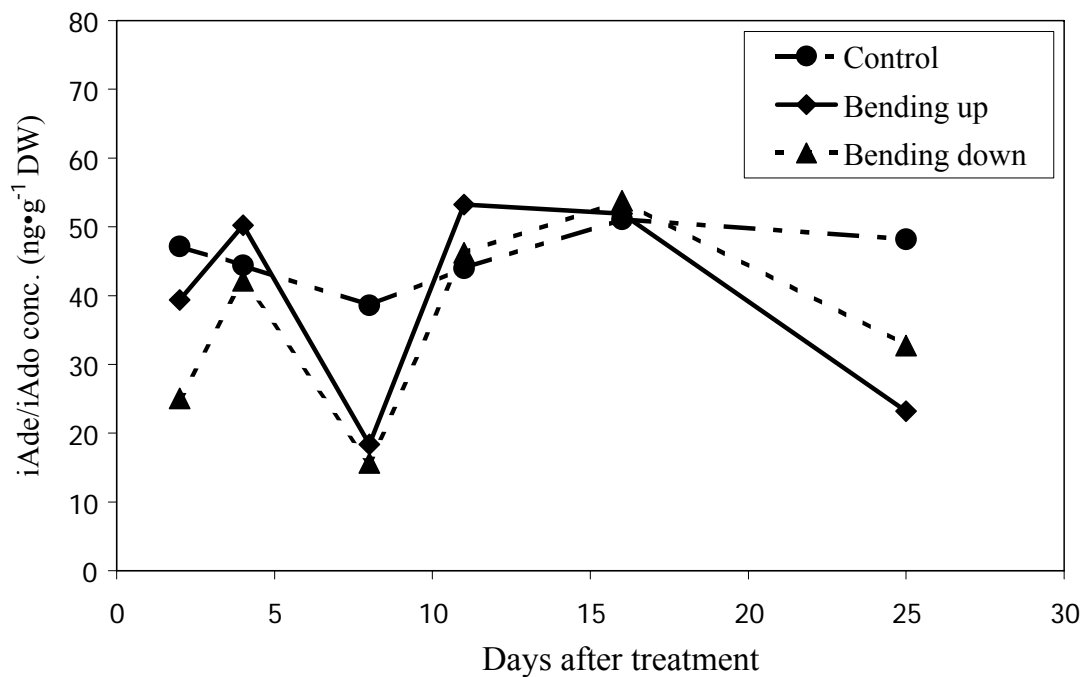


Figure 4.6. iAde/iAdo concentrations in the apical portion of the shoot of cv. Elstar after bending treatments

#### 4.1.1.2 Gibberellins

During the first half period of sampling concentrations of GA showed no differences among treatments in all three cultivars. After that bending up treatment seemed to decrease GA concentrations in all three cultivars as compared to bend down treatment and control. Surprisingly bending down treatment increased GA concentrations at the last sampling date in all three cultivars (Figure 4.7, 4.8 and 4.9), possibly because the tips of these shoots started regrowth and bend up again.



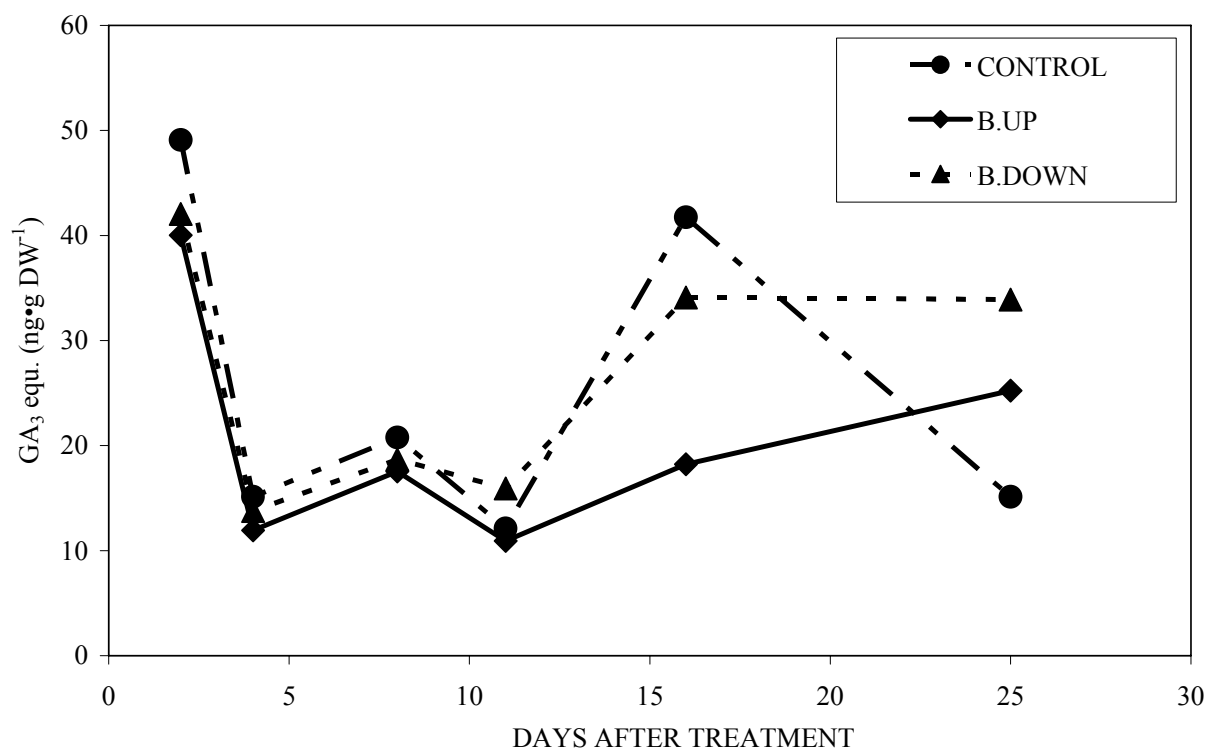


Figure 4.7. GA concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

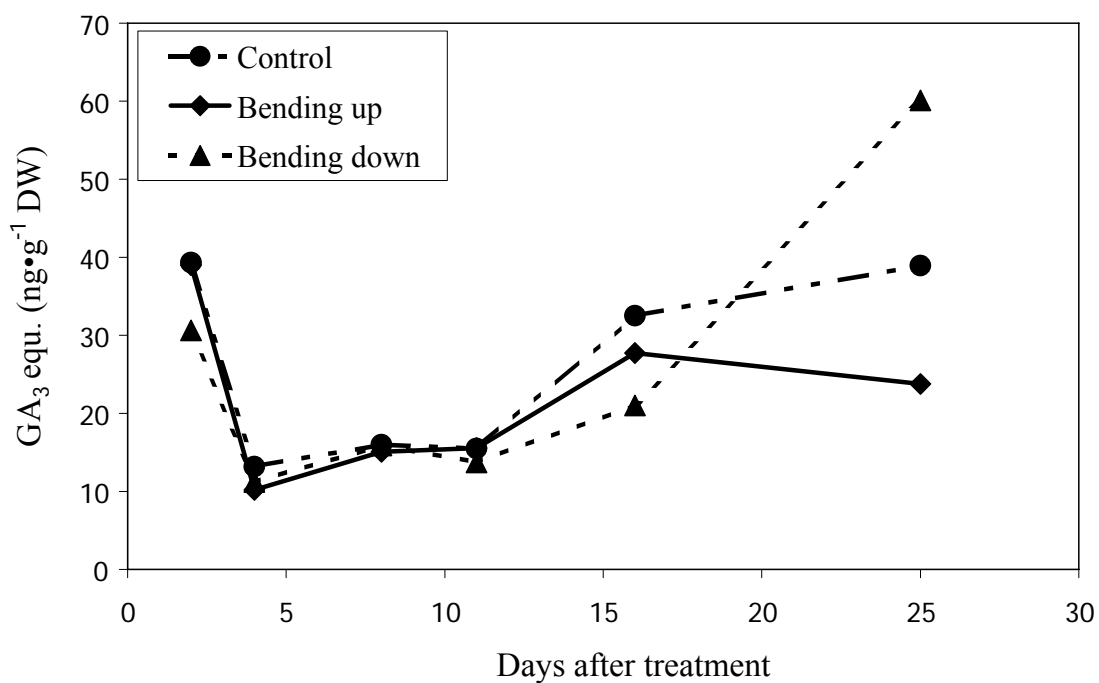


Figure 4.8. GA concentrations in the apical portion of the shoot of cv. Boskoop after bending treatments

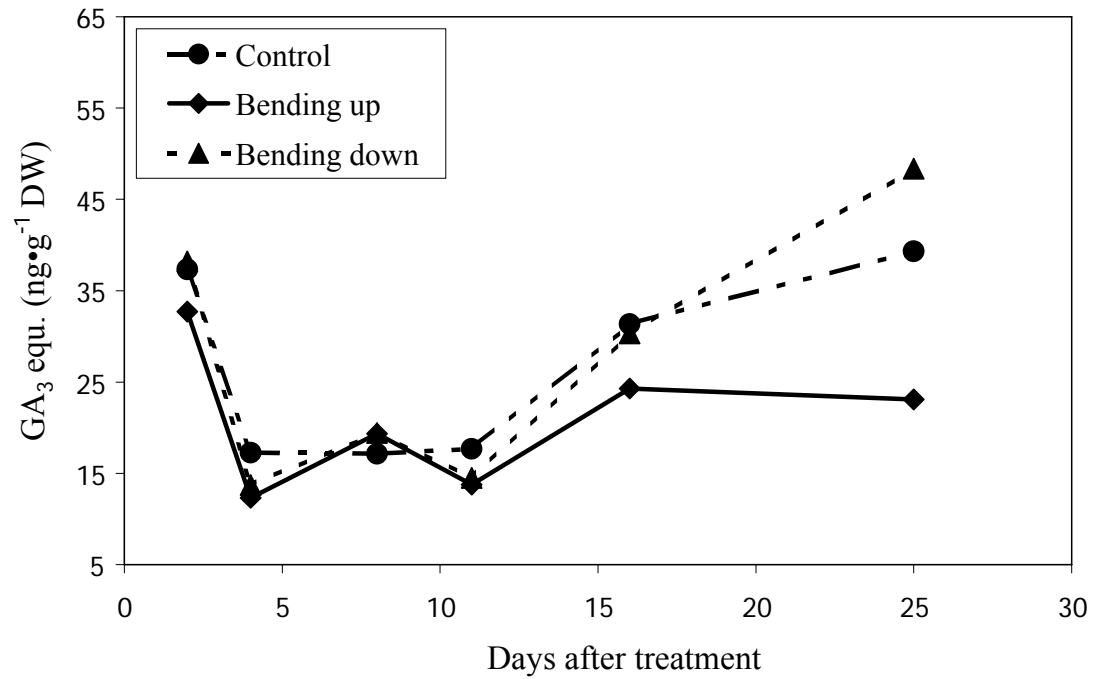


Figure 4.9. GA concentrations in the apical portion of the shoot of cv. Elstar after bending treatments

#### 4.1.1.3 IAA

IAA concentrations were not different until at 11 DAT. After that bending up and bending down treatments decreased IAA concentrations in cv. Golden Delicious (Figure 4.10), Boskoop (Figure 4.11) and Elstar when compared to control (Figure 4.12). Furthermore bending up treatment seemed to have stronger influence in cvs. Boskoop and Elstar.

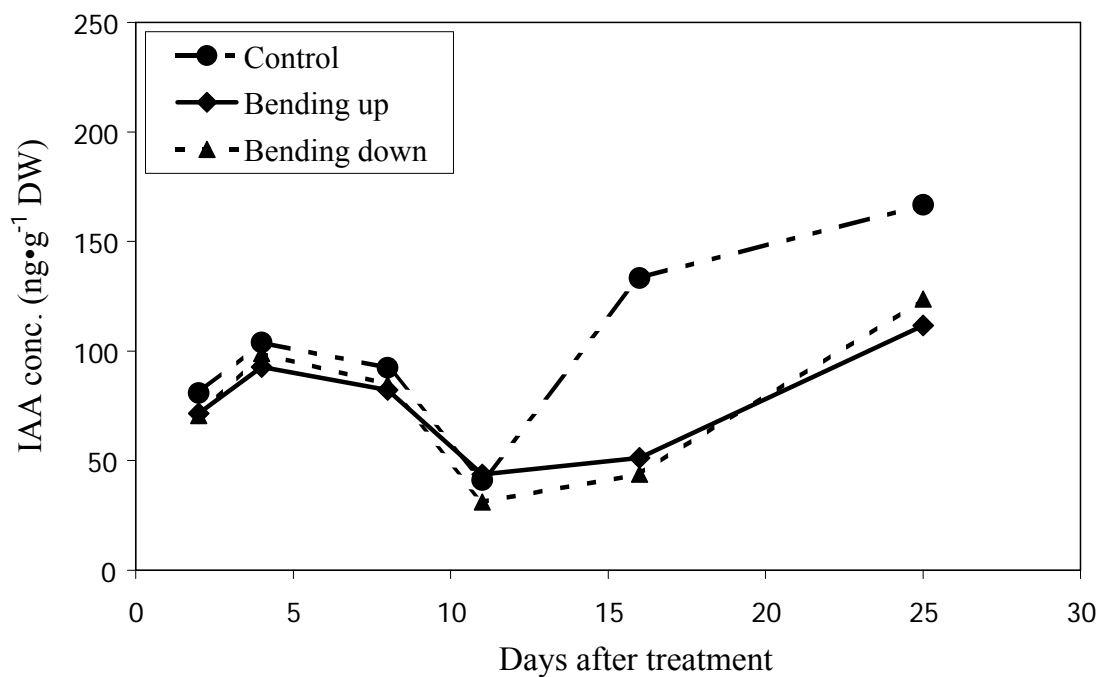


Figure 4.10. IAA concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatment

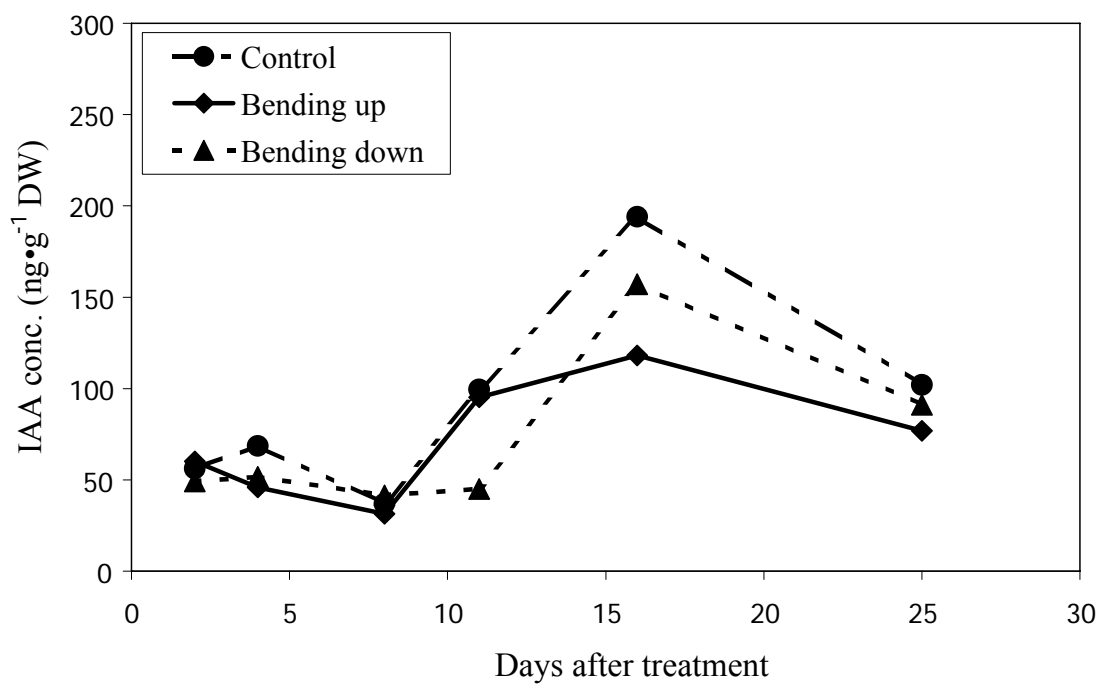


Figure 4.11. IAA concentrations in the apical portion of the shoot of cv. Boskoop after bending treatments

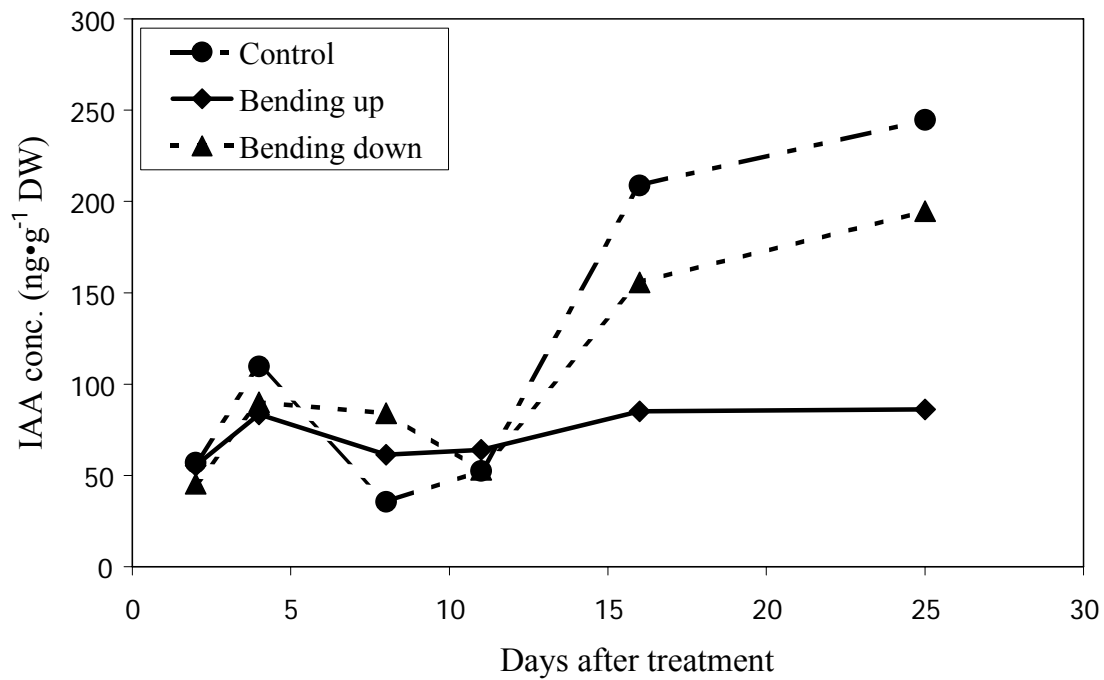


Figure 4.12. IAA concentrations in the apical portion of the shoot of cv Elstar after bending treatments

#### 4.1.1.4 ABA

ABA concentrations were in general not different among treatments, except at the last sampling date of cv. Golden Delicious where bending up shoots seemed to contain more ABA than control and bending down treatments (Figure 4.13). All three treatments exhibited a peak between 10 and 20 days after treatment without a clear differentiation between control and bending.

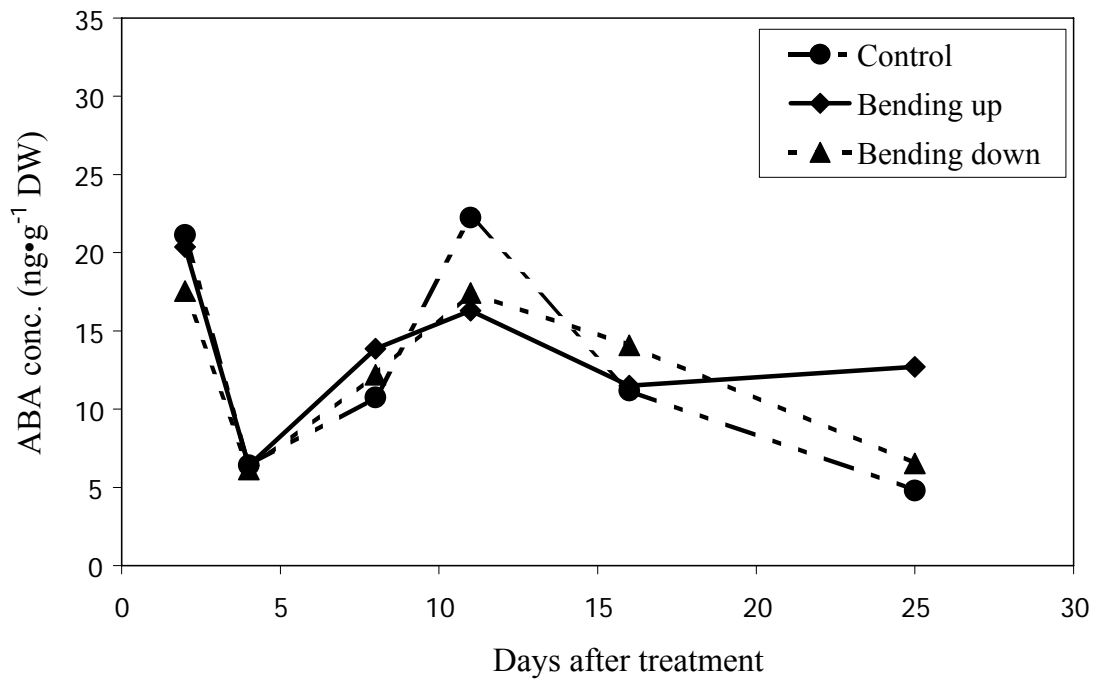


Figure 4.13. ABA concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

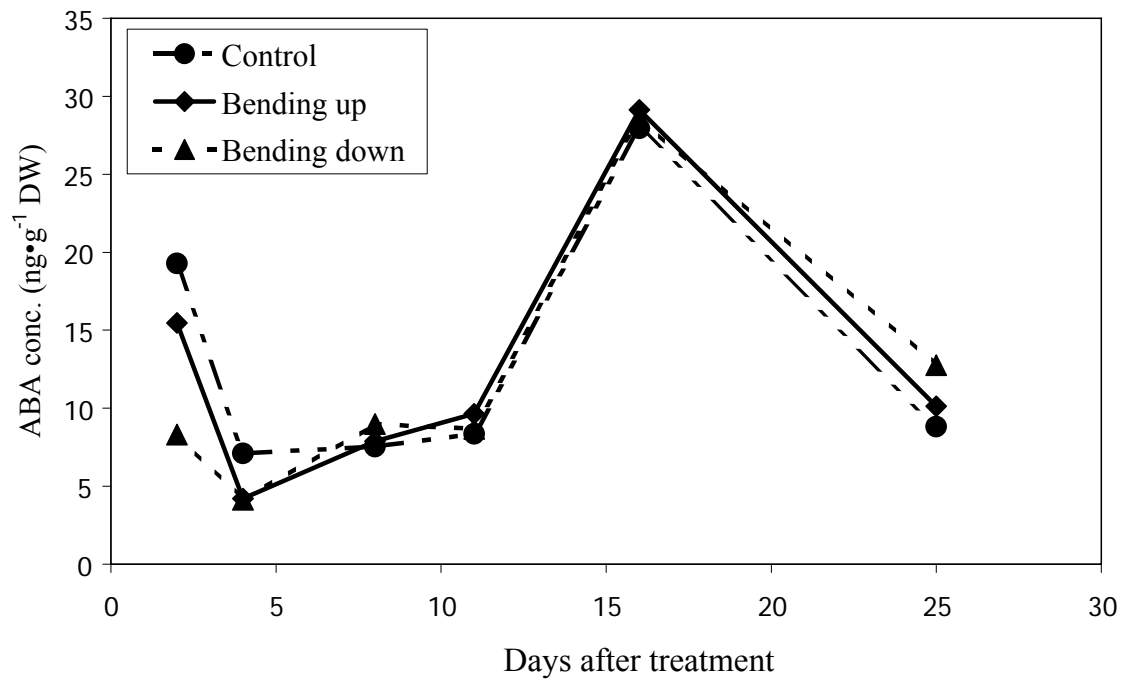


Figure 4.14. ABA concentrations in the apical portion of the shoot of cv. Boskoop after bending treatments

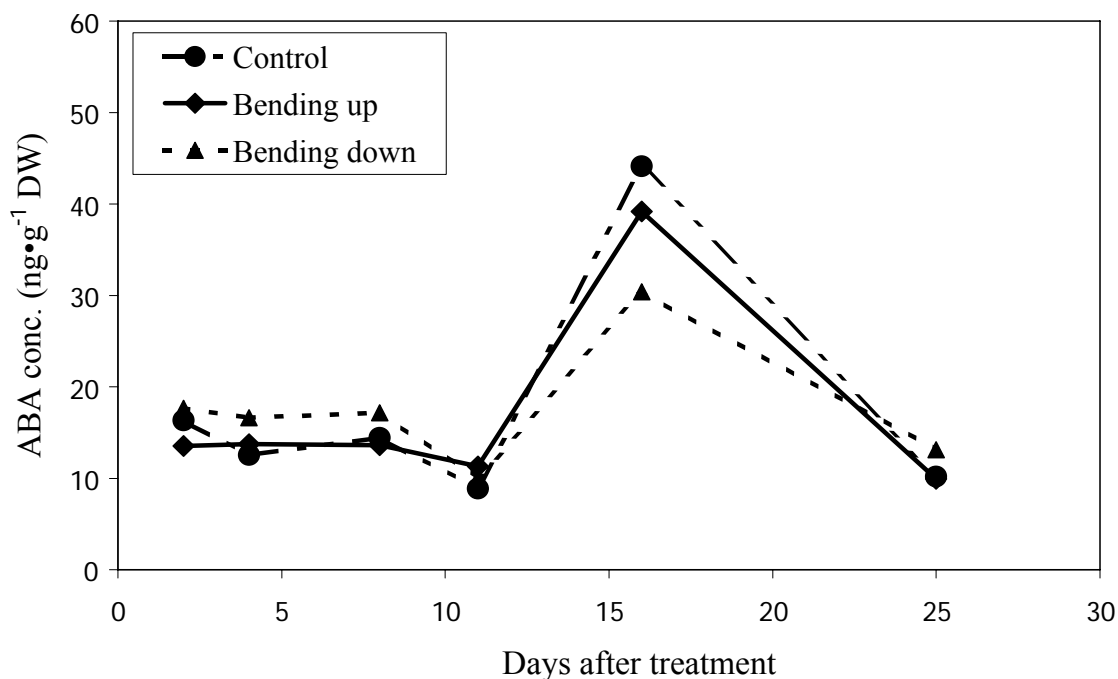


Figure 4.15. ABA concentrations in the apical portion of the shoot of cv. Elstar after bending treatments

#### 4.1.2 Effect of bending on hormonal changes in shoots and leaves of apple seedling cv. Golden Delicious

##### 4.1.2.1 Cytokinins

Z/ZR concentrations in shoots and leaves of apple seedlings decreased considerably after bending down treatment as compared to control with the exception of one peak in the leaves that was higher than the control (Figure 4.16). IAde/iAdo concentrations, which decreased sharply at the beginning, were not different in both shoots and leaves (Figure 4.17).

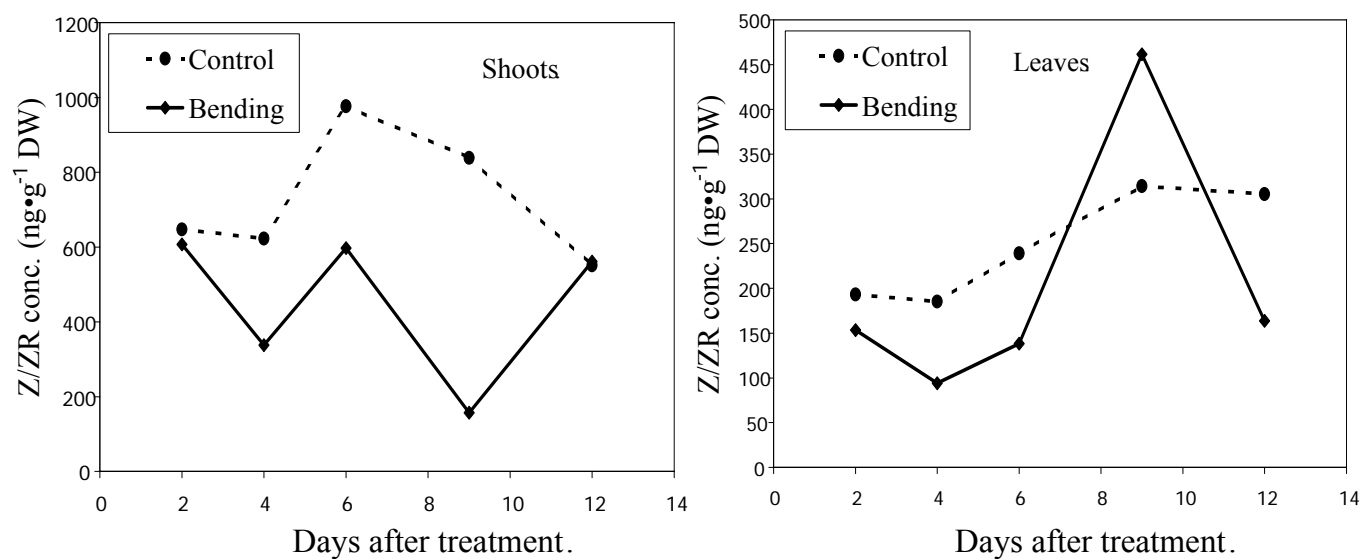


Figure 4.16. Z/ZR in the apical part of the shoots and leaves of apple seedling of cv. Golden Delicious after bending treatment

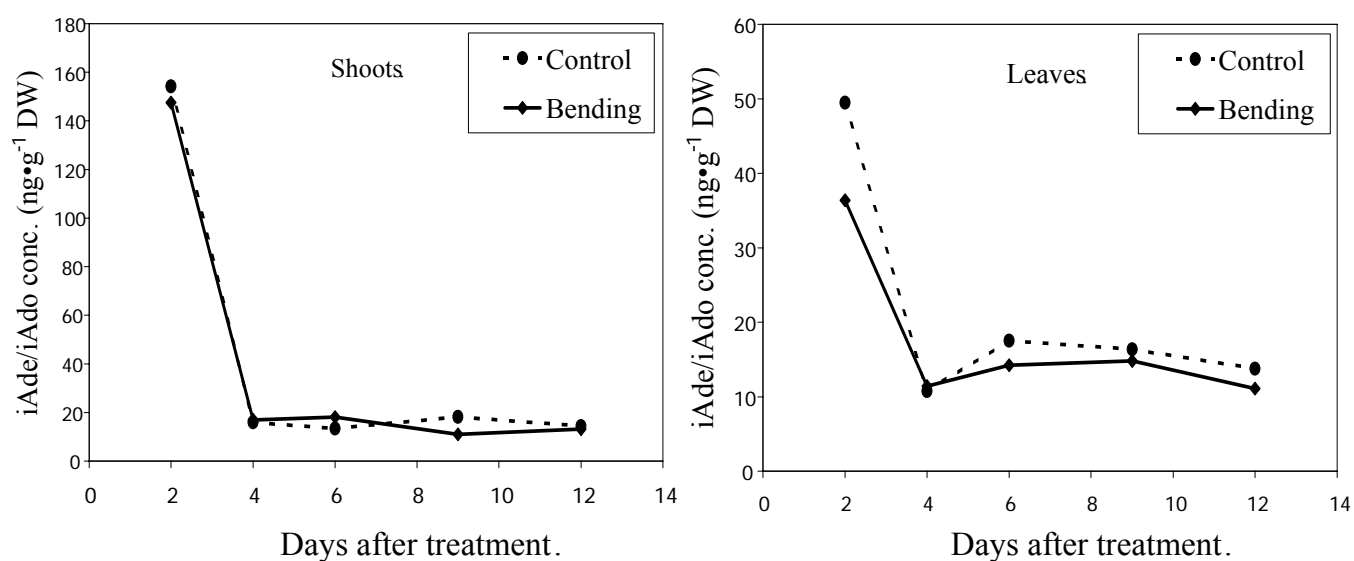


Figure 4.17. iAde/iAdo in the apical part of the shoot and leaf of apple seedling of cv. Golden Delicious after bending treatment

#### 4.1.2.2 Gibberellins

In shoot and leaves GA concentrations in bended shoots are almost ever lower than in control plants (Figure 4.18).

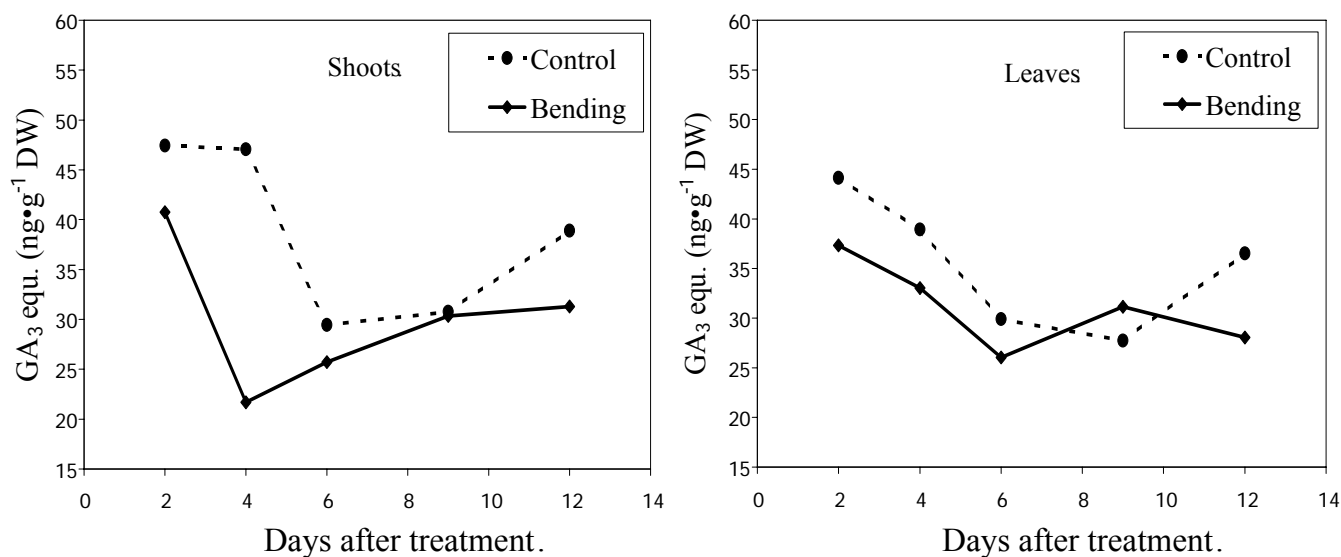


Figure 4.18. GAs in the apical part of the shoot and leave of apple seedling of cv. Golden Delicious after bending treatment

#### 4.1.2.3 IAA

IAA concentrations were reduced in shoots after bending treatment. Contrary, there were higher IAA concentrations in leaves after bending (Figure 4.19).



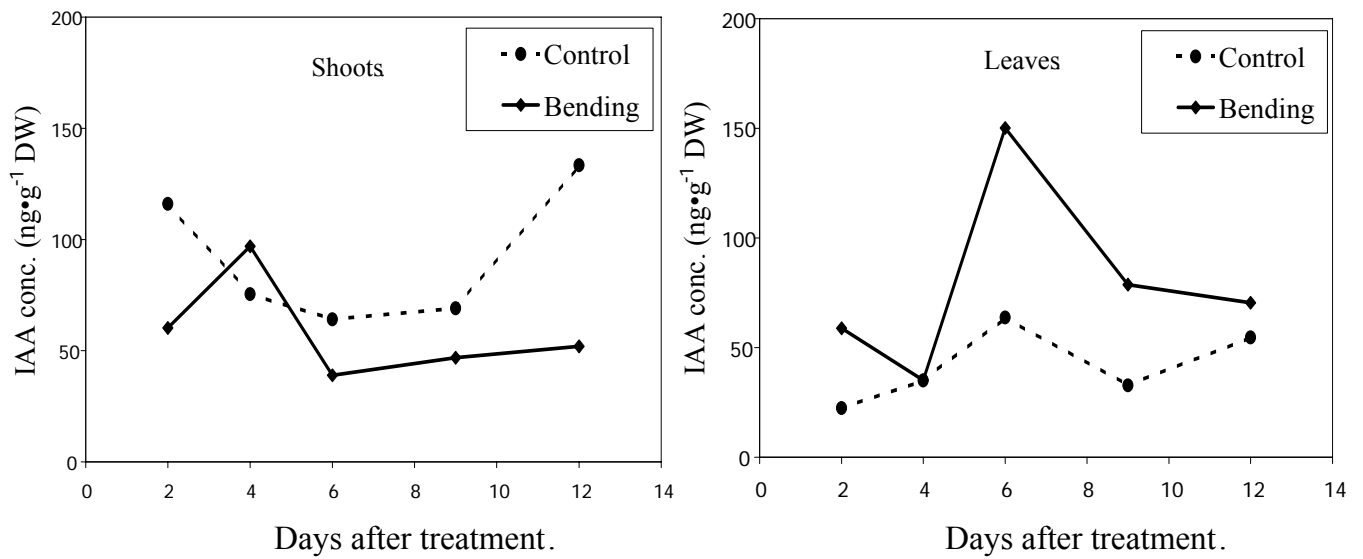


Figure 4.19. IAA in the apical part of the shoot and leave of apple seedling of cv. Golden Delicious after bending treatment

#### 4.1.3 Effect of Alar plus Ethrel treatment on hormonal changes in growing and non-growing shoots of apple trees

##### 4.1.3.1 Golden Delicious

##### 4.1.3.1.1 Cytokinins

Z/ZR as well as iAde/iAdo concentrations seemed to increase more after spraying with Alar and Ethrel both in growing and non-growing shoots as compared to control shoots (Figure 4.20 and 4.21).

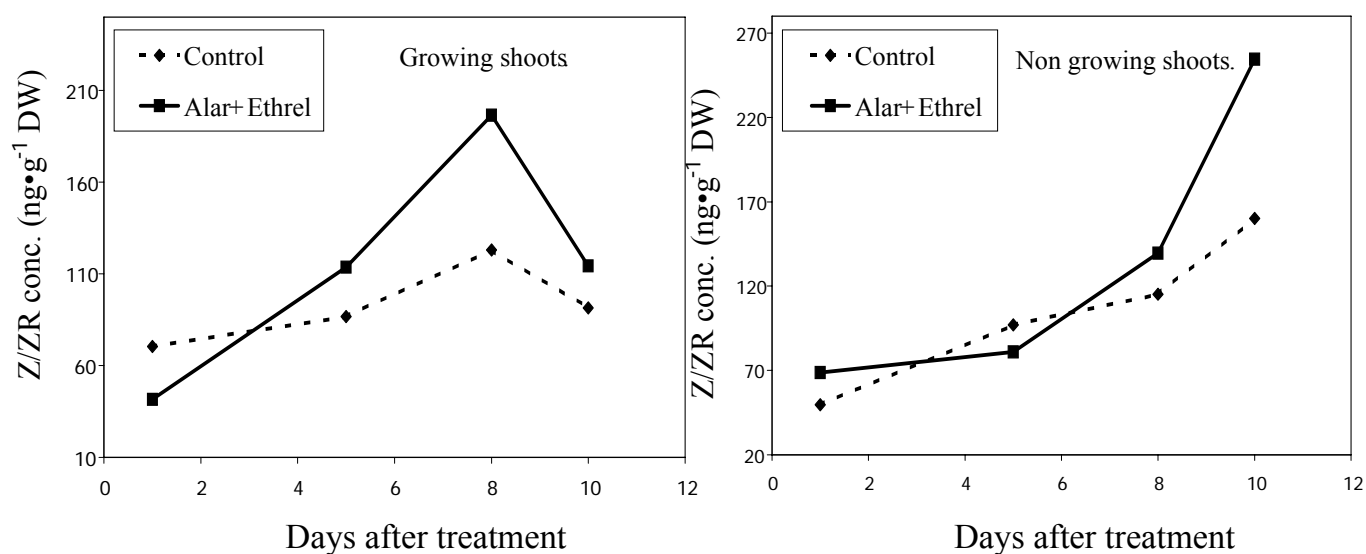


Figure 4.19. Z/ZR concentrations in growing and non-growing shoots of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

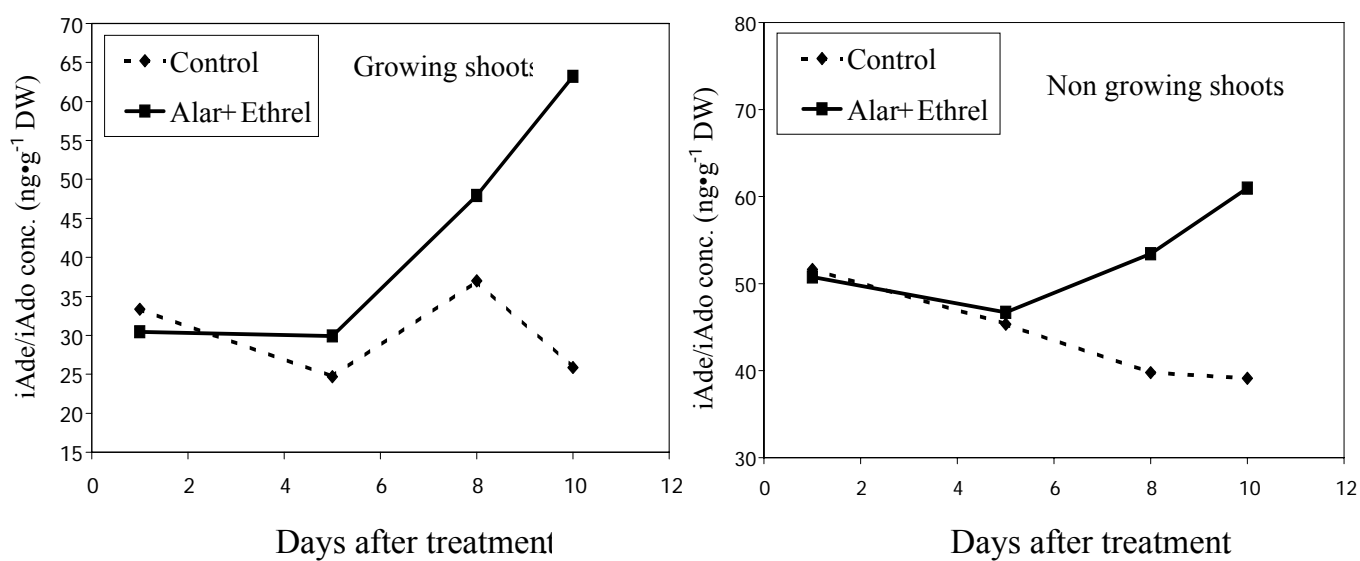


Figure 4.20. iAde/iAdo concentrations in growing and non-growing shoots of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

#### 4.1.3.1.2 Gibberellins

GA concentrations in growing shoots were somewhat higher at the first half of the sampling period after spraying with Alar plus Ethrel. After that it was lower than control, however there was no difference at the last sampling date. The treatment decreased GA concentrations in non-growing shoots at the last half of the sampling period (Figure 4.21).

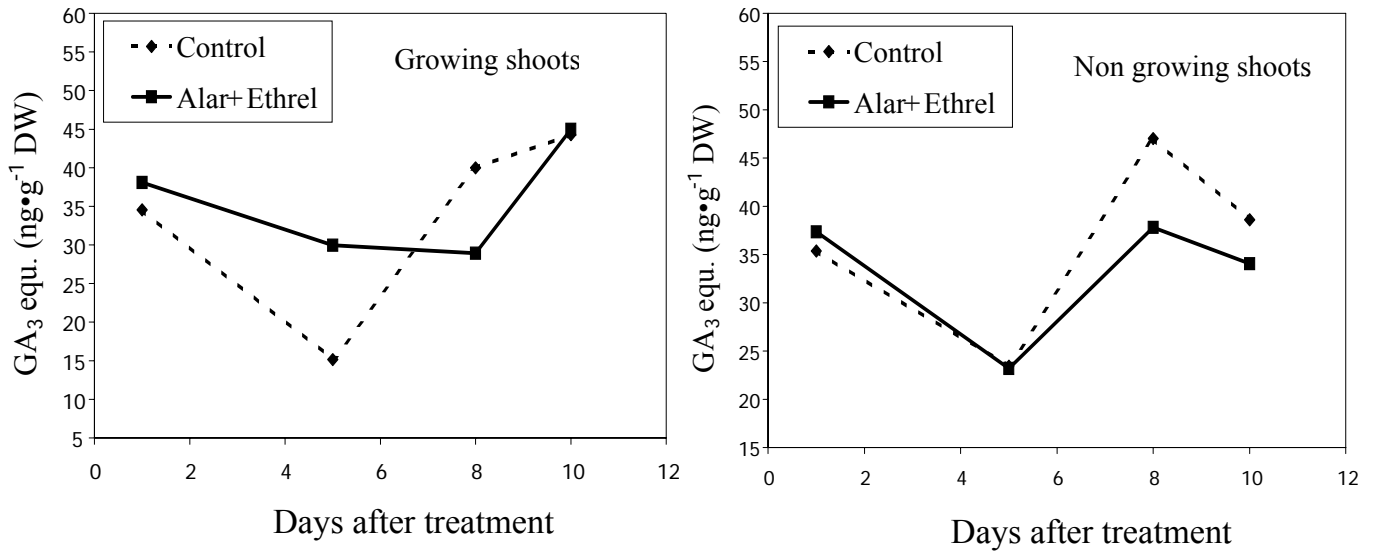


Figure 4.21. GA concentrations in growing and non-growing shoot of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

#### 4.1.3.1.3 IAA

Spraying with a combination of Alar plus Ethrel decreased the IAA concentration in growing shoots. In non-growing shoots, however, a clear tendency was not found (Figure 4.22)

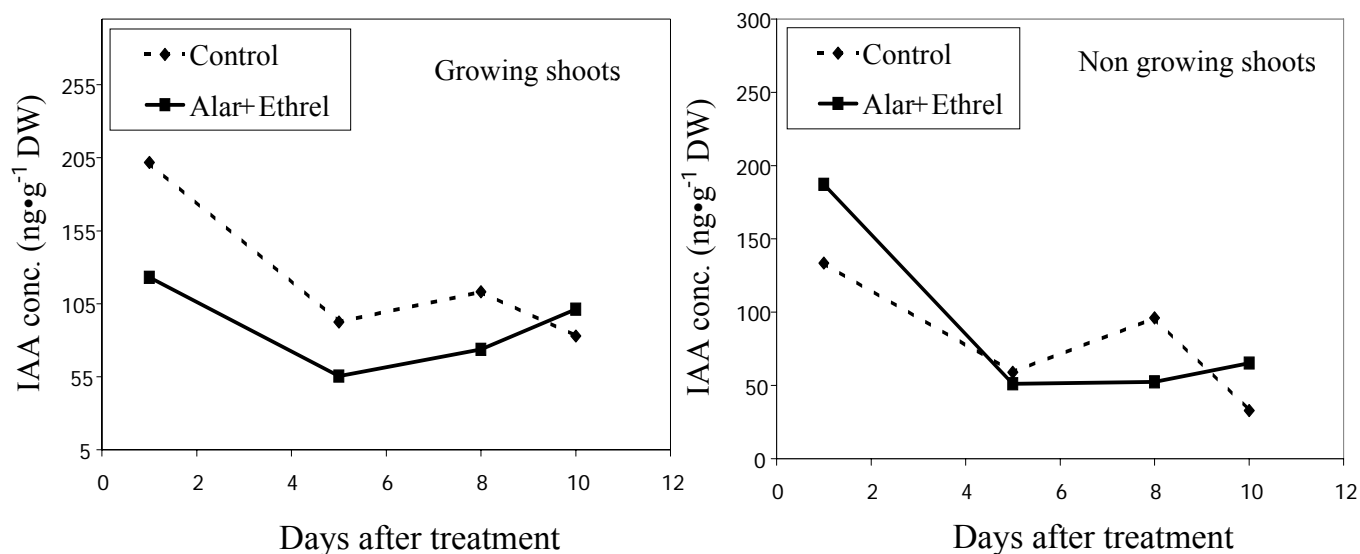


Figure 4.22. IAA concentrations in growing and non-growing shoot of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

#### 4.1.3.1.4 ABA

ABA concentrations were not markedly different throughout the sampling period. (Figure 4.23)

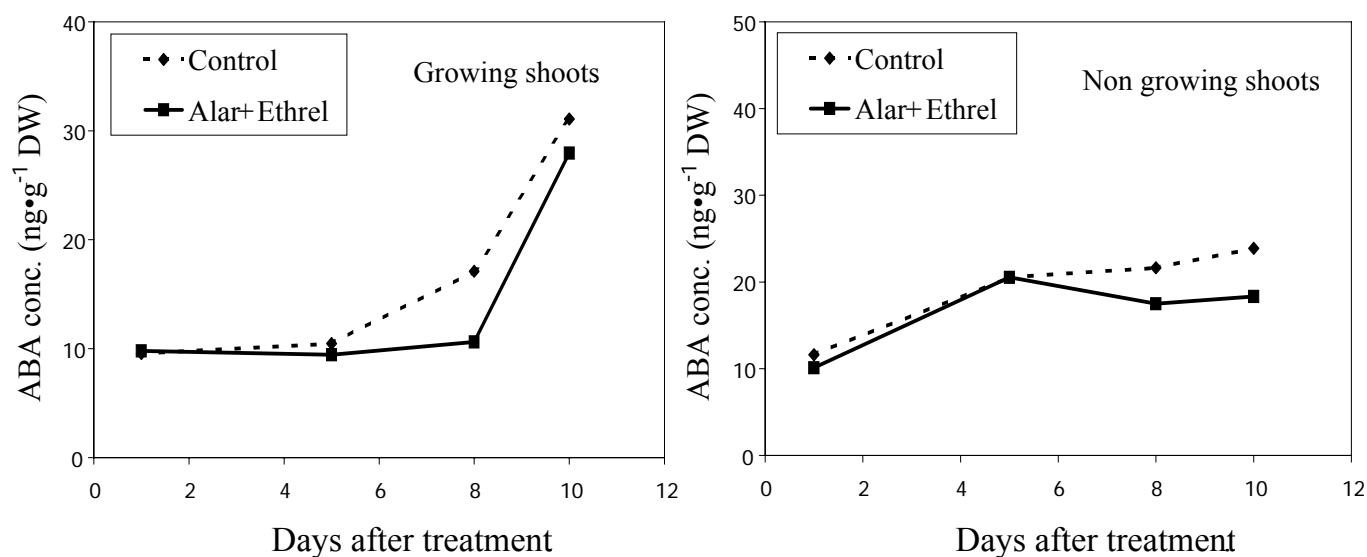


Figure 4.23. ABA concentrations in growing and non-growing shoot of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

#### 4.1.3.2 Boskoop

##### 4.1.3.2.1 Cytokinins

Contrary to the cv. Golden Delicious, CK concentrations were slightly lower in both growing and non-growing shoots after spraying with Alar plus Ethrel as compared to the control (Figure 4.24, 3.25).

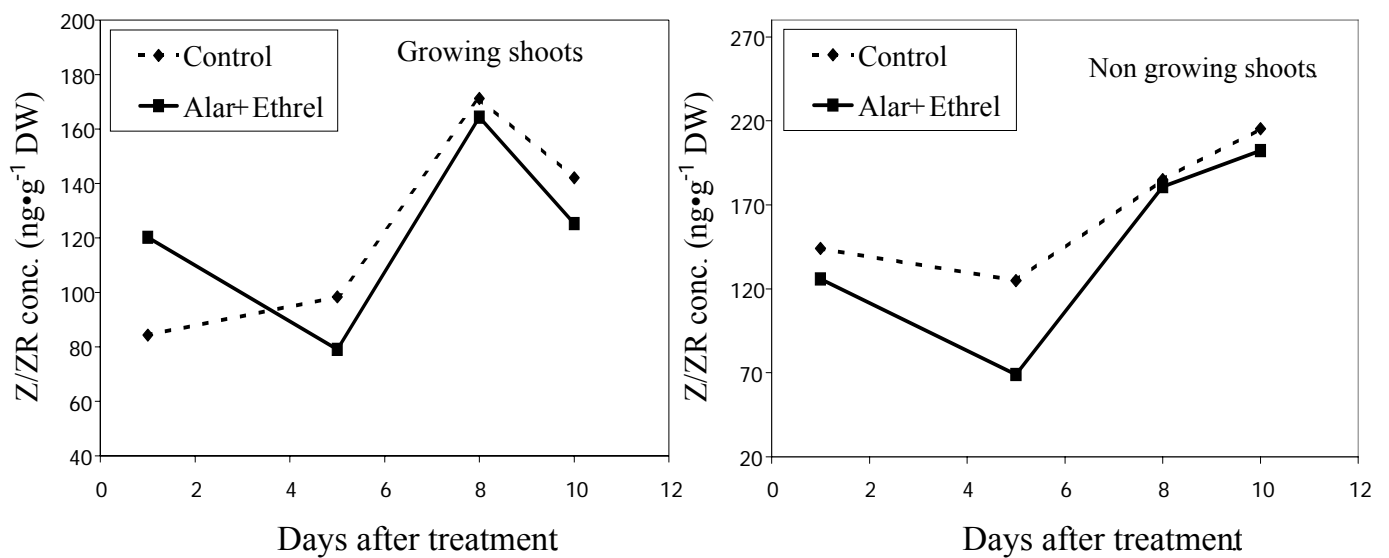


Figure 4.24. Z/ZR concentrations in growing and non-growing shoot of apple trees of cv. Boskoop after spraying with Alar plus Ethrel

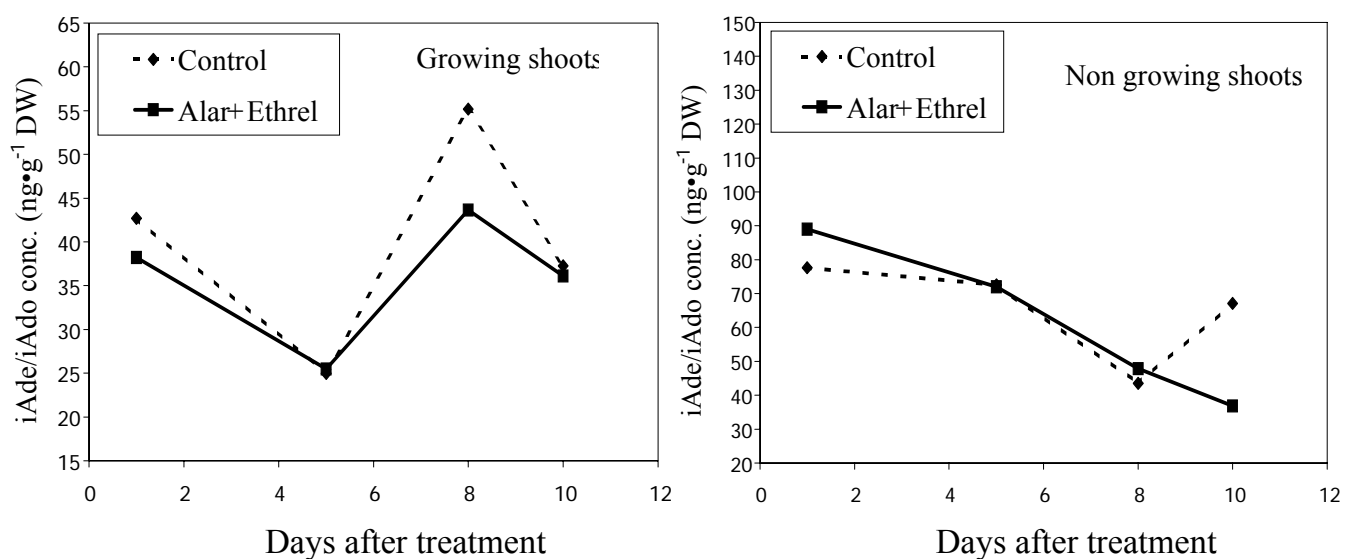


Figure 4.25. iAde/iAdo concentrations in growing and non-growing shoot of apple trees of cv. Boskoop after spraying with Alar plus Ethrel

#### 4.1.3.2.2 Gibberellins

GA concentrations in growing shoots were not such different between sprayed and control trees. However in non-growing shoots the GA concentrations were reduced at the first half of the sampling period (Figure 3.26).

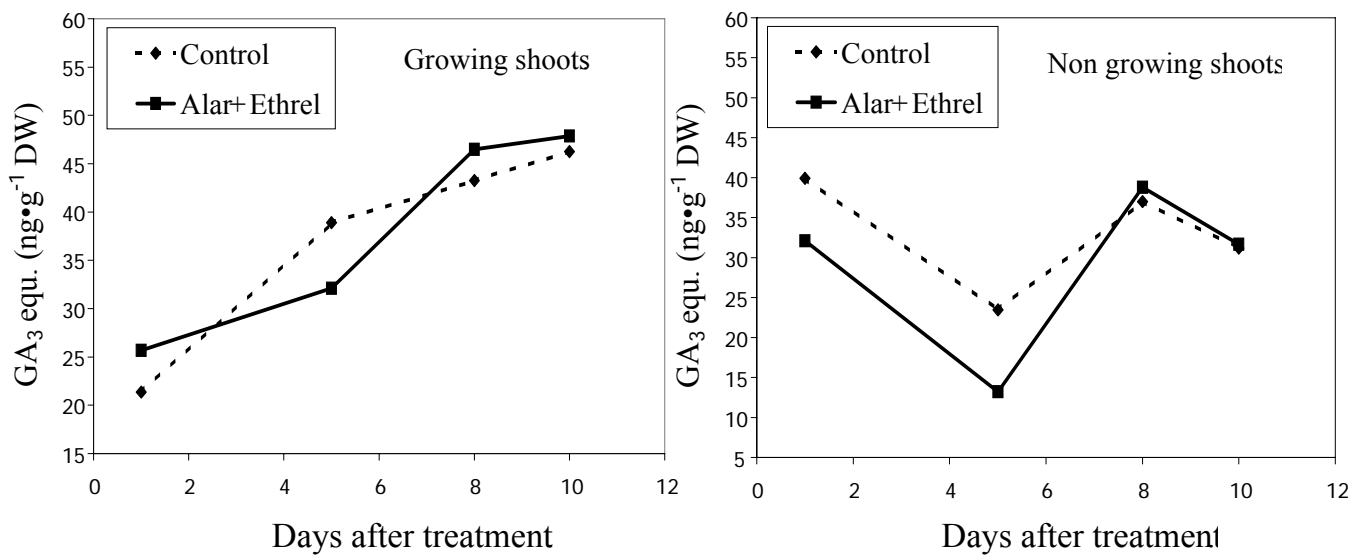


Figure 4.26. GA concentrations in growing and non-growing shoots of apple trees of cv. Boskoop after spraying with Alar plus Ethrel

#### 4.1.3.2.3 IAA

IAA concentrations in non-growing shoots were with one exception not different between sprayed and control trees, whereas in growing shoots no clear picture can be seen after spraying Alar plus Ethrel (Figure 4.27)

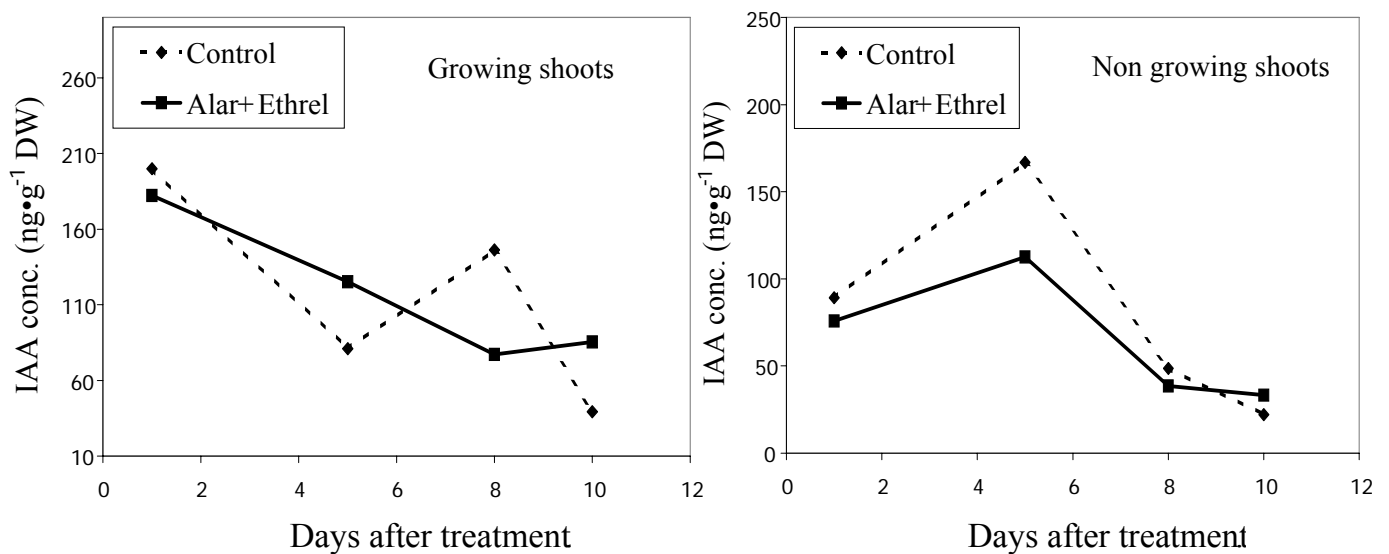


Figure 4.27. IAA concentrations in growing and non-growing shoot of apple trees of cv. Boskoop after spraying with Alar plus Ethrel

## 4.1.3.2.4 ABA

Similar to cv. Golden Delicious, spraying Alar plus Ethrel seemed to decrease ABA concentrations in growing and non-growing shoots but the results are too erratic for a clear statement (Figure 4.28)

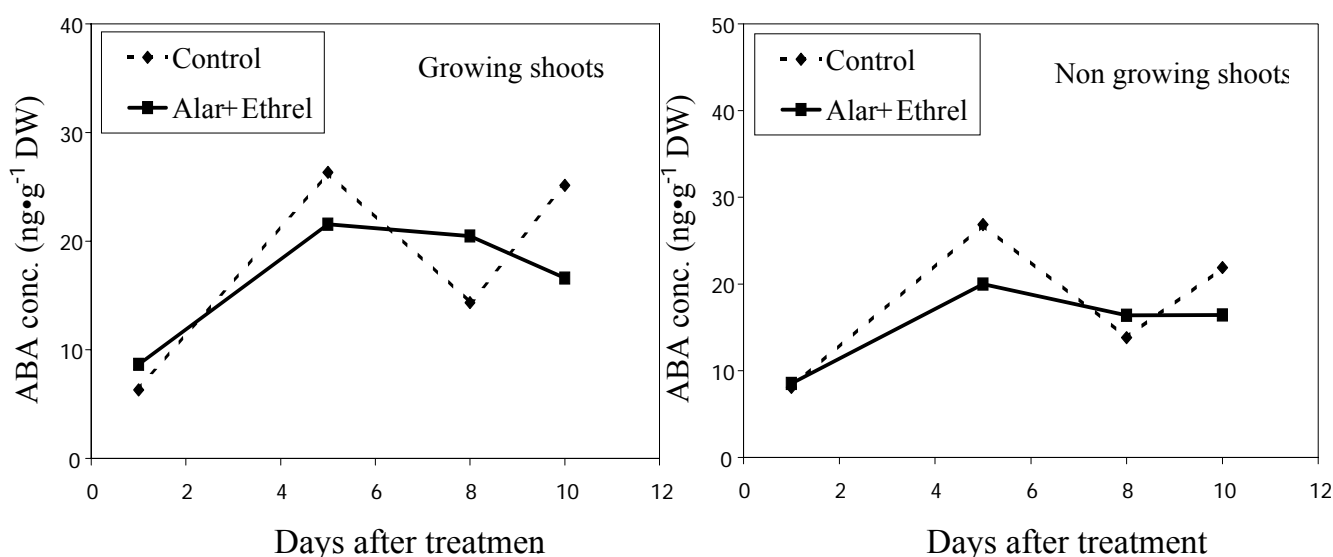


Figure 4.28. ABA concentrations in growing and non-growing shoot of apple trees of cv. Boskoop after spraying with Alar plus Ethrel

## 4.1.3.2.5 Ethylene

Due to the nature of Ethrel as a “synthetic precursor” for ethylene the generally considerable increase in ethylene production in treated trees of both cvs. is of no surprise (Figure 4.29 and 4.30). Also the larger difference between treated and control trees during the first about 6 days after treatment was to be expected.



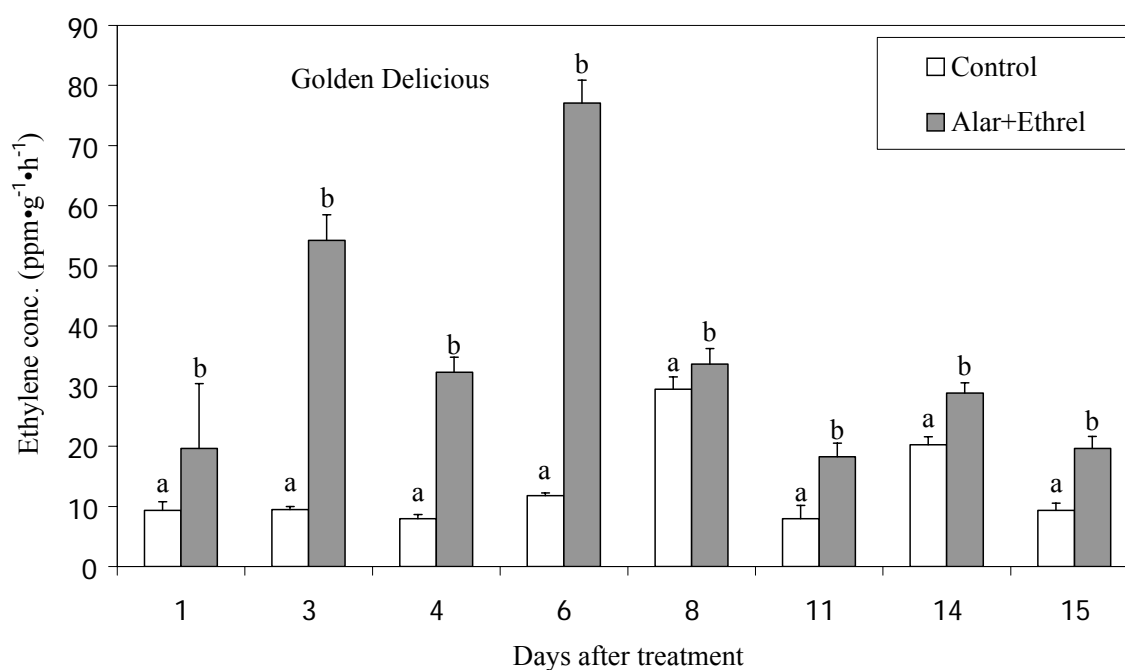


Figure 4.29. Ethylene concentration in apical portion of the shoot of cv. Golden Delicious after spraying with Alar plus Ethrel

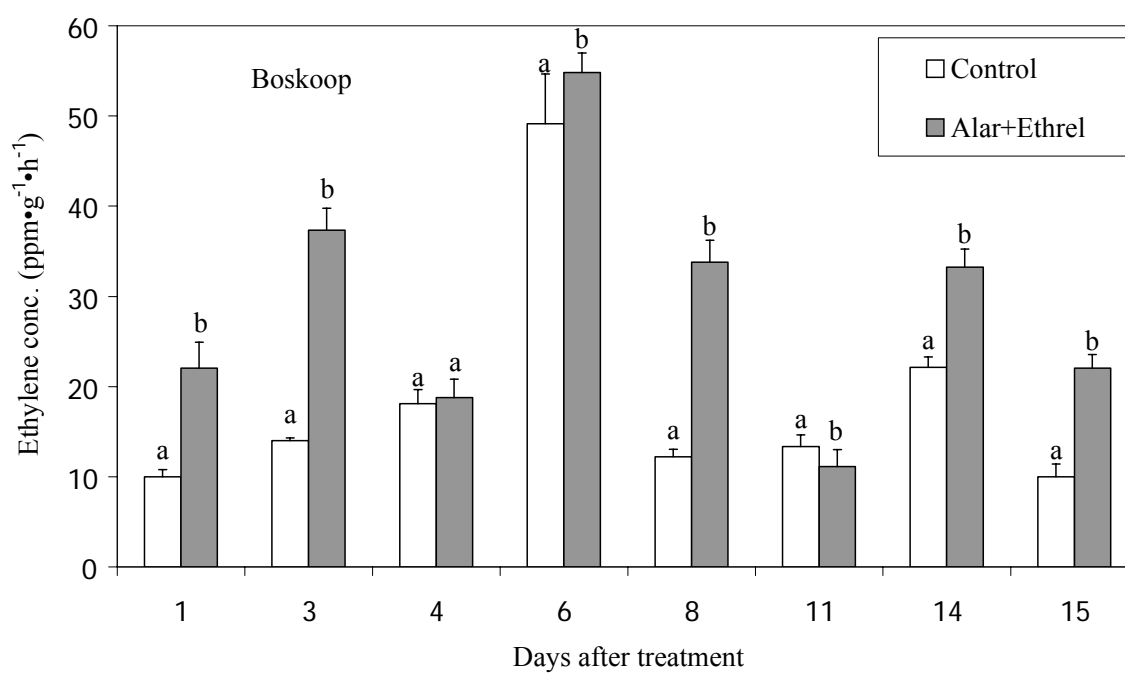


Figure 4.30. Ethylene concentration in apical portion of the shoot of cv. Boskoop after spraying with Alar plus Ethrel

## 4.2 Experiments in 2002

### 4.2.1 Effect of bending on hormonal changes in the apical portion of apple tree shoots

#### 4.2.1.1 Cytokinins

Z/ZR concentrations in apple shoots of the cv. Golden Delicious seemed lower at the first half of sampling period after bending treatments. After that the Z/ZR concentrations raised to higher level than control (Figure 4.31). Surprisingly bending treatments decreased Z/ZR concentrations in apple shoots of cv. Idared throughout the sampling period (Figure 4.32)

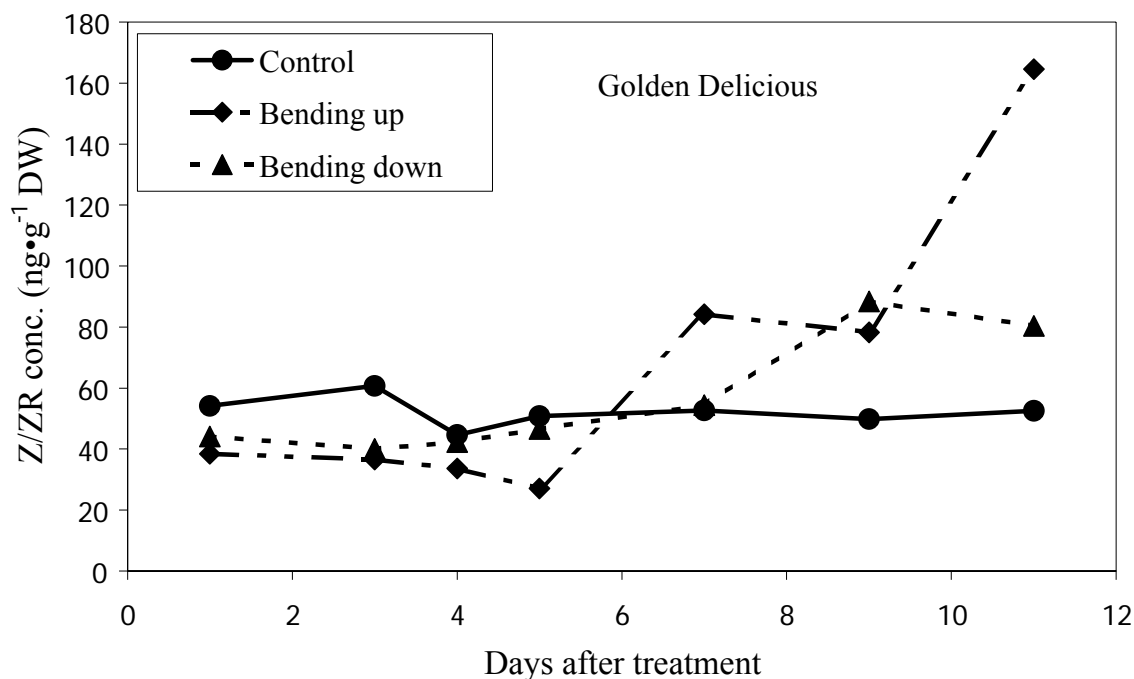


Figure 4.31. Z/ZR concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

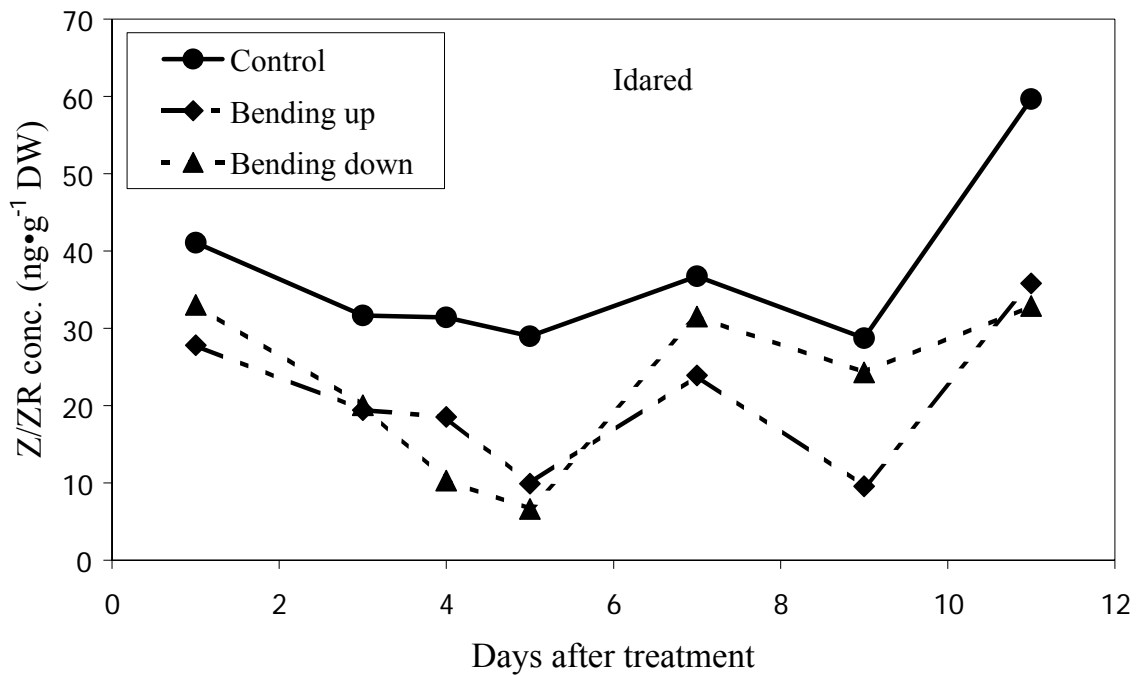


Figure 4.32. Z/ZR concentrations in the apical portion of the shoot of cv. Idared after bending treatments

In addition bending treatment seemed to decrease iAde/iAdo concentrations in apple shoots of cv. Golden Delicious until at 4 DAT later on the values raised above control levels in the bending up treatment. Contrary to that the bending down treatment caused the concentration to decrease as compared to the control, except the last sampling date (Figure 4.33). In cv. Idared, the iAde/iAdo concentrations were not so different between control and treatments, except again at the last sampling date, where higher concentrations were observed after bending treatments (Figure 4.34).

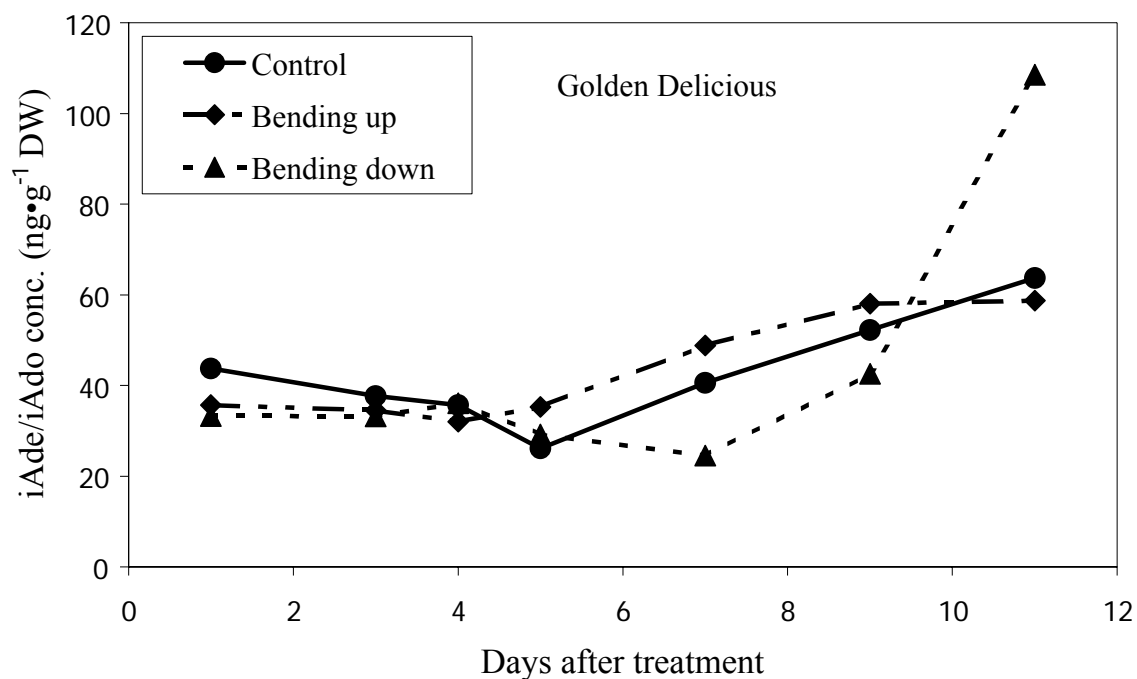


Figure 4.33. iAde/iAdo concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

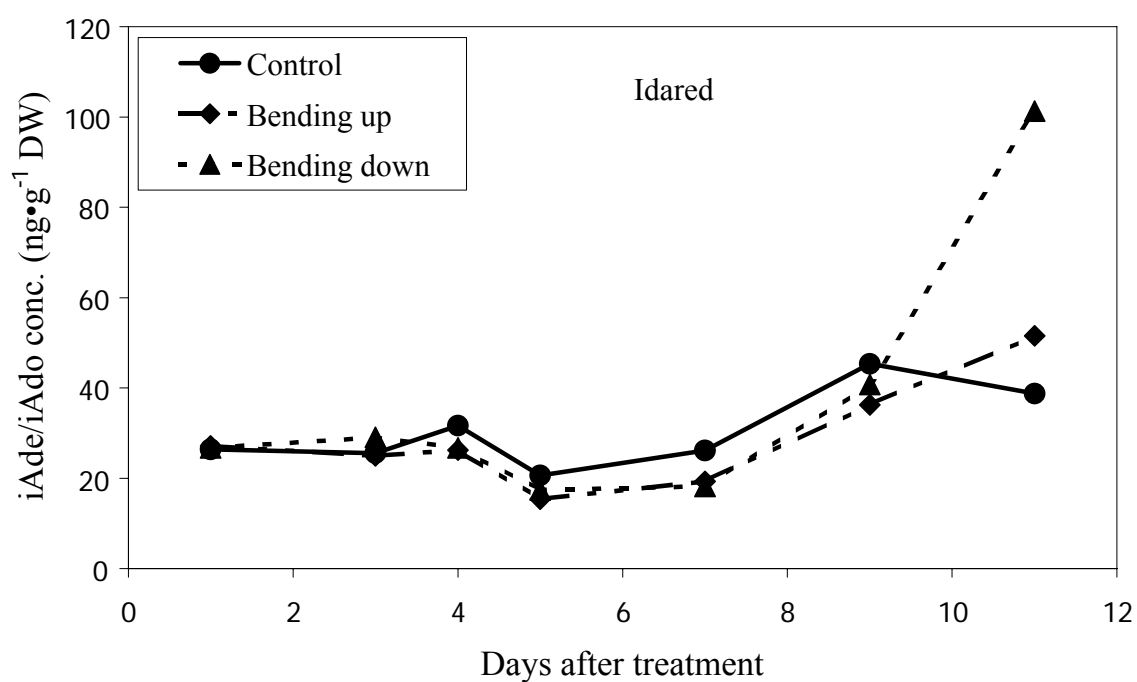


Figure 4.34. iAde/iAdo concentrations in the apical portion of the shoot of cv. Idared after bending treatments

## 4.2.1.2 Gibberellins

GA concentrations in apple shoots of cv. Golden Delicious seemed lower at 1 and 3 days after bending treatments as compared to control. Thereafter the reverse was observed (Figure 4.35). There were small differences between control and treatments in cv. Idared (Figure 4.36).

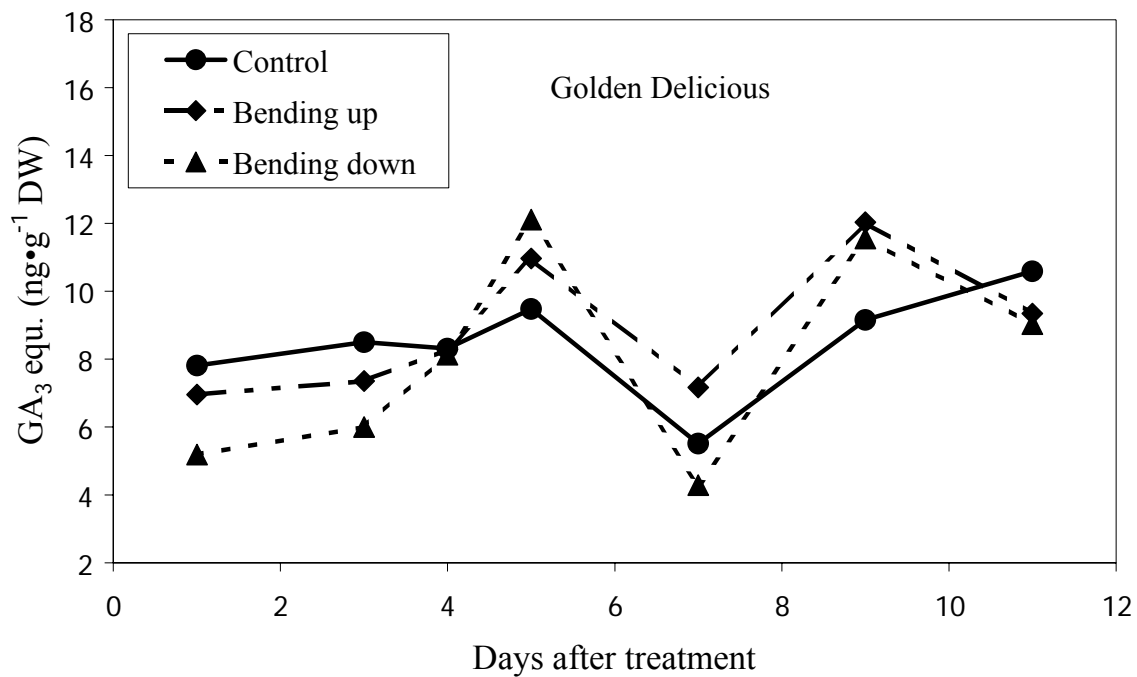


Figure 4.35. GA concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

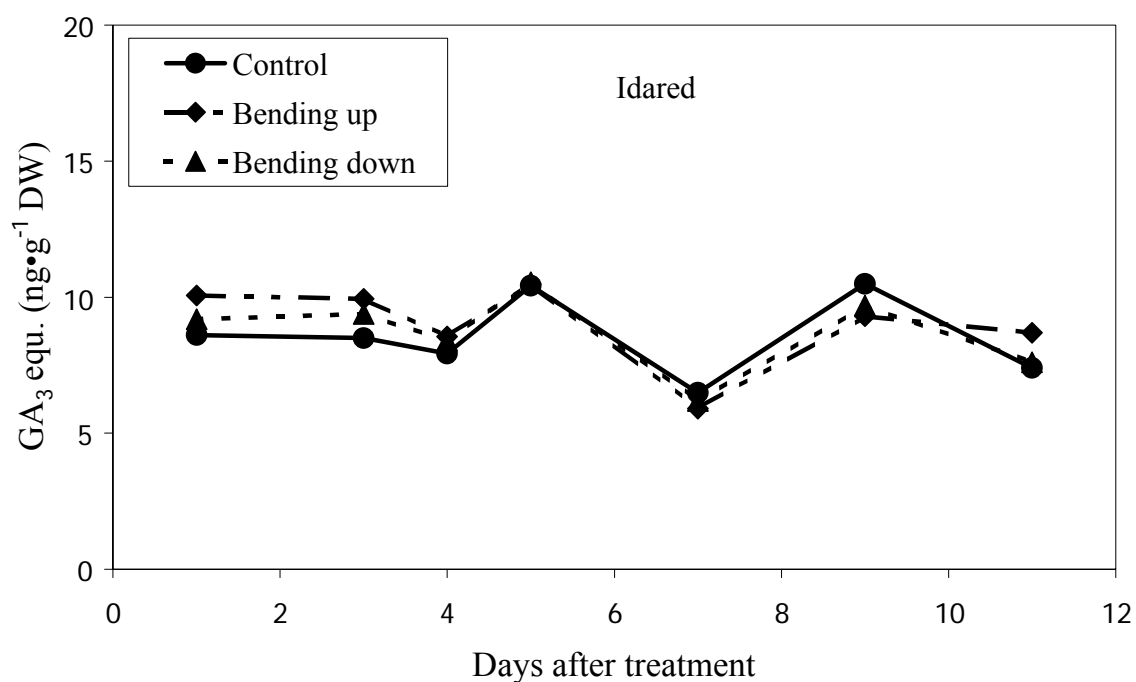


Figure 4.36. GA concentrations in the apical portion of the shoot of cv. Idared after bending treatments

#### 4.2.1.3 IAA

Both Bending up and bending down treatments decreased IAA concentrations in apple shoots of the cv. Idared (Figure 4.38). This was true in cv. Golden Delicious at 4 until 11 days after bending up treatment and at 1, 5, 9 and 11 days after bending down treatment (Figure 4.37).

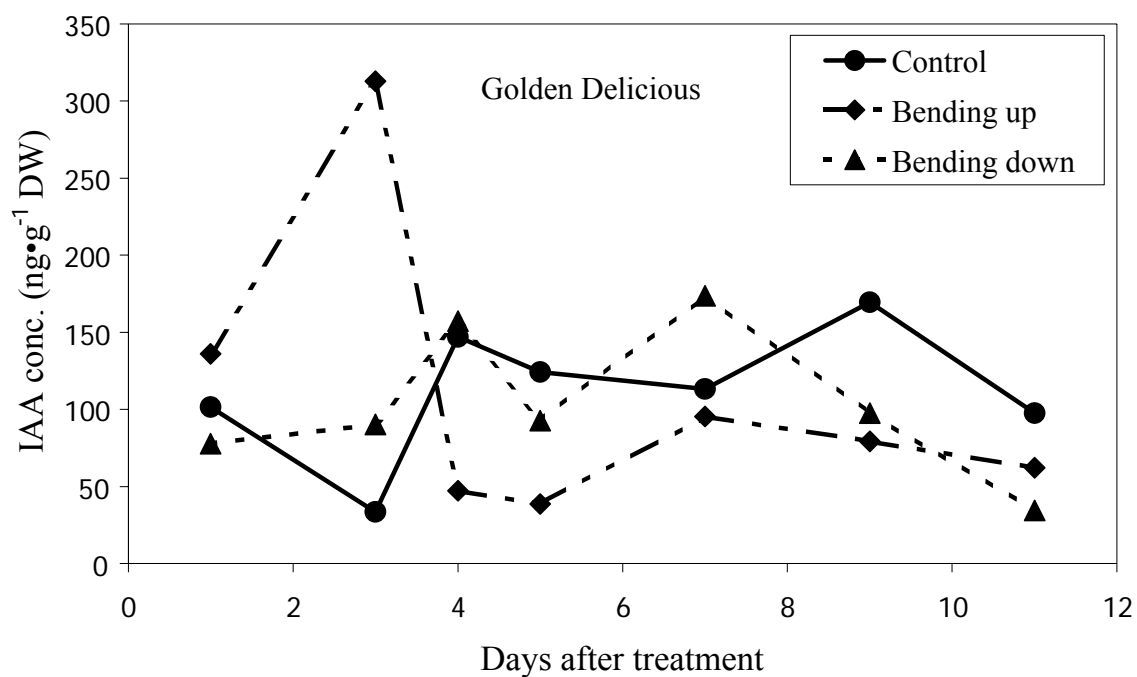


Figure 4.37. IAA concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments

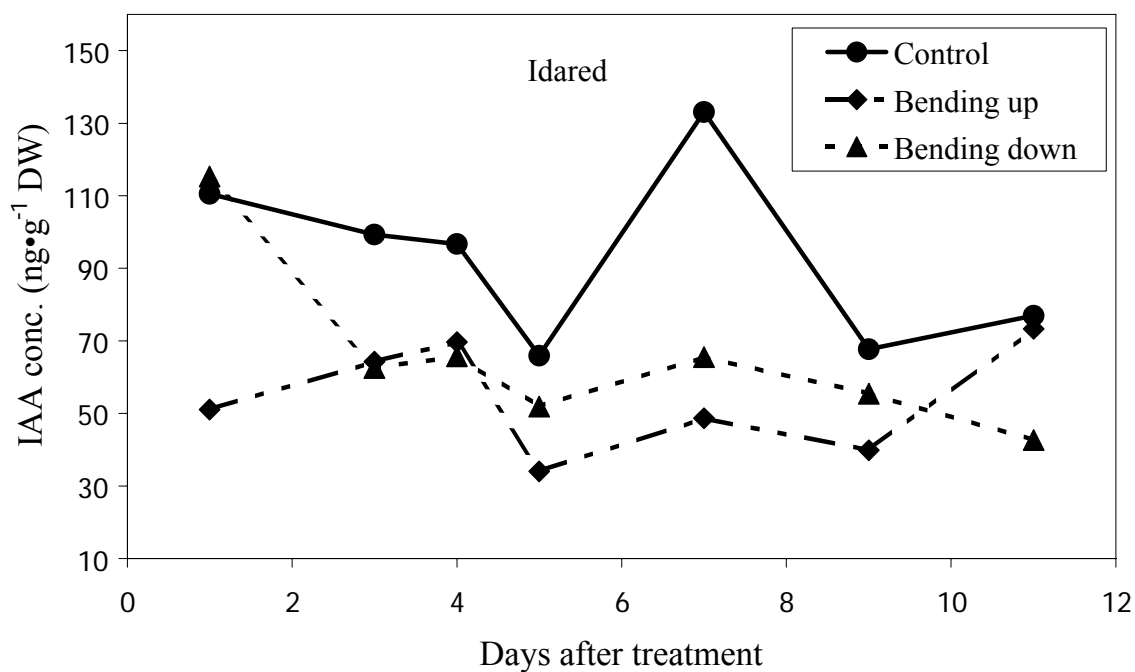


Figure 4.38. IAA concentrations in the apical portion of the shoot of cv. Idared after bending treatments

## 4.2.1.4 ABA

There were no clear tendencies of ABA concentrations among treatments in apple shoot of the cv. Golden Delicious (Figure 4.39). However the bending down treatment generally increased the ABA concentration in apple shoots of cv. Idared (Figure 4.40)

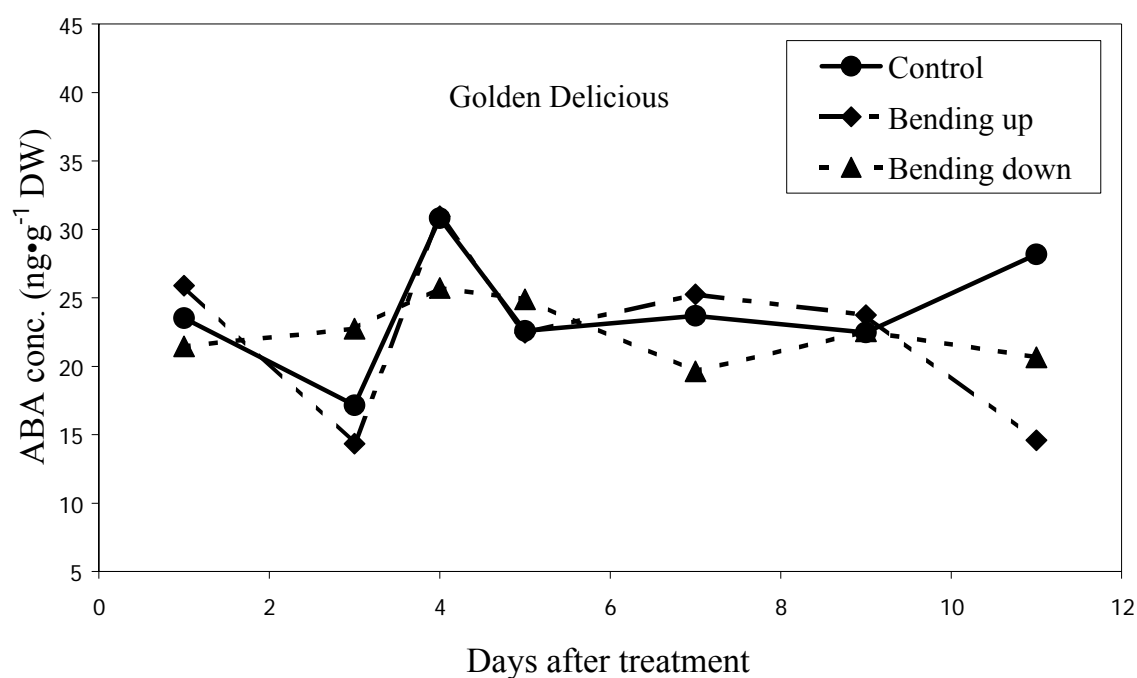


Figure 4.39. ABA concentrations in the apical portion of the shoot of cv. Golden Delicious after bending treatments



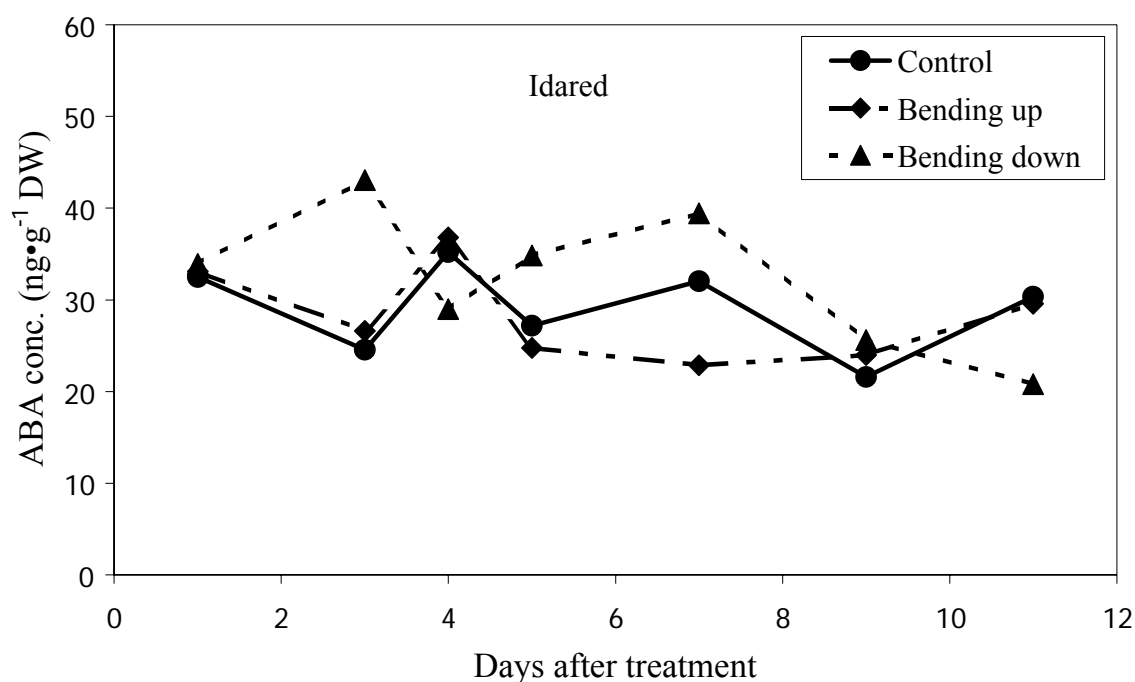


Figure 4.40. ABA concentrations in the apical portion of the shoot of cv. Idared after bending treatments

#### 4.2.2 Effect of Alar plus Ethrel on hormonal changes in growing shoots of apple trees

##### 4.2.2.1 Cytokinins

Spraying Alar plus Ethrel generally increased Z/ZR concentrations in growing shoot of cv. Golden Delicious. This is also true for cv. Idared except for day 2 after treatment (Figure 4.42)

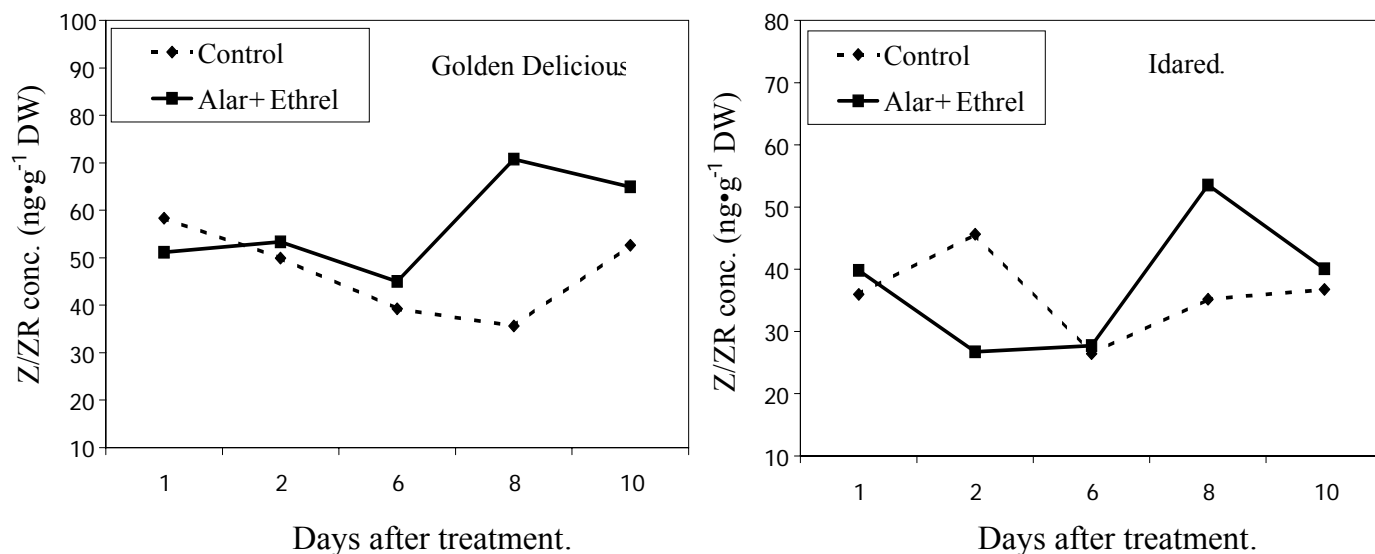


Figure 4.41. Z/ZR concentrations in growing shoots of apple trees of cv. Golden Delicious and Idared after spraying with Alar plus Ethrel

Alar plus Ethrel spraying increased Z/ZR concentrations in wood and bark during 2-3 days after spraying as compared to control, then the concentrations declined to the same level as the control (Figure 4.42)

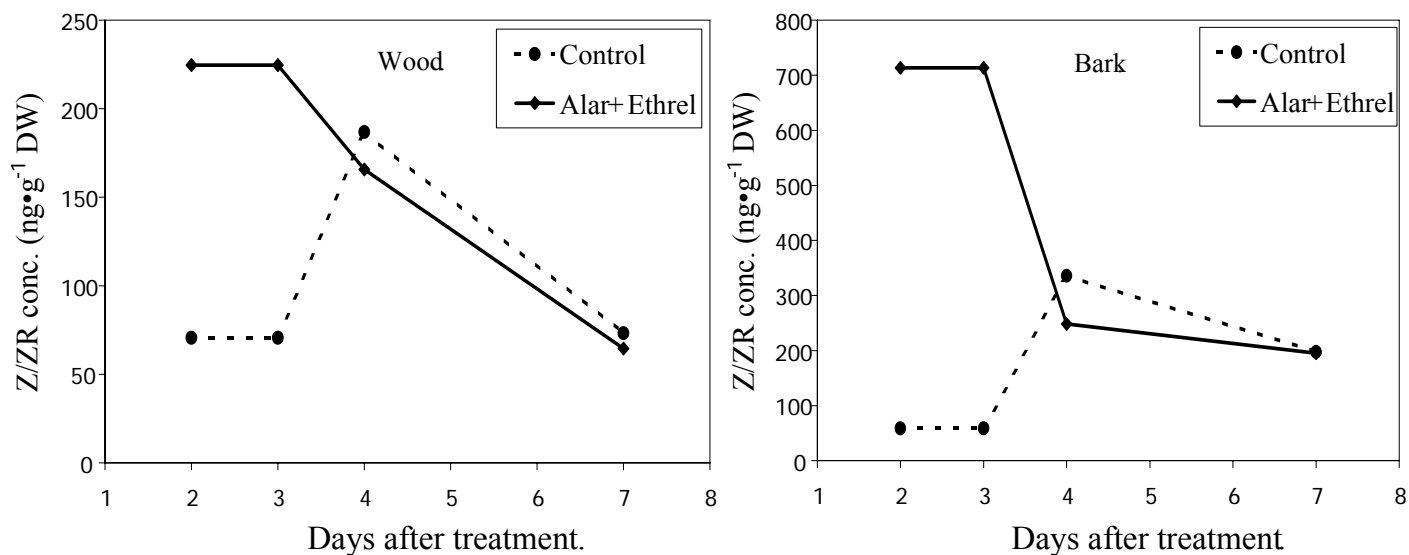


Figure 4.42. Z/ZR concentrations in wood and bark of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

iAde/iAdo concentrations in growing shoots of both cultivars were lower at the beginning after spraying then they increased slightly being higher than control at the last sampling date (Figure 4.43). There were no clear tendencies of iAde/iAdo concentrations in wood and bark (Figure 4.44).

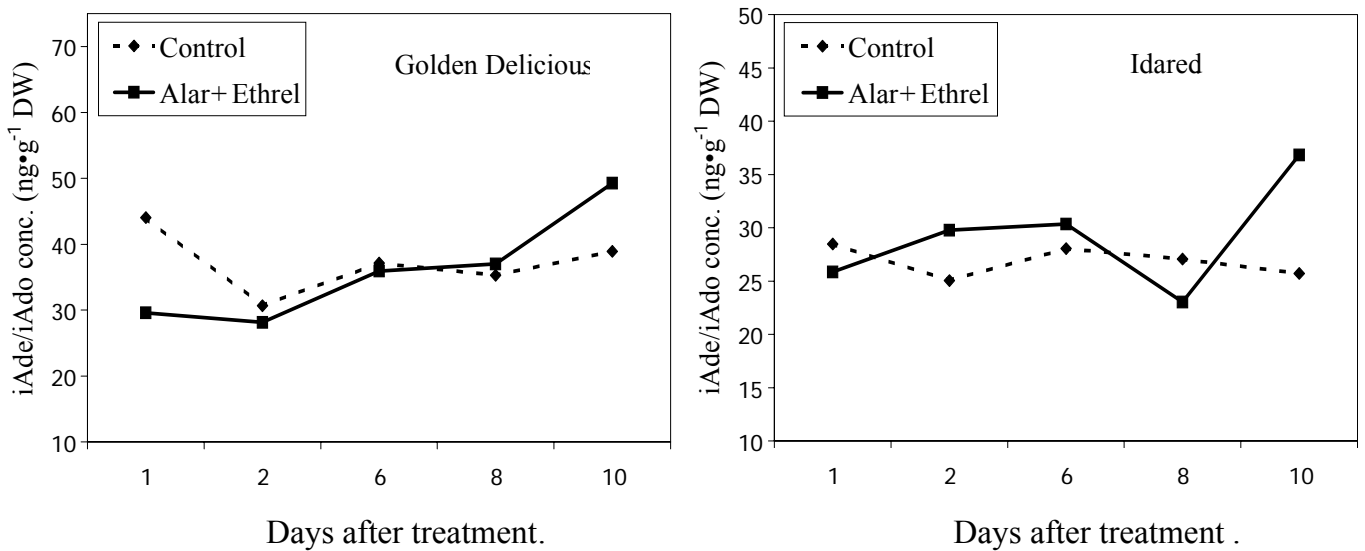


Figure 4.43. iAde/iAdo concentrations in growing shoots of apple trees of cv. Golden Delicious and Idared after spraying with Alar plus Ethrel

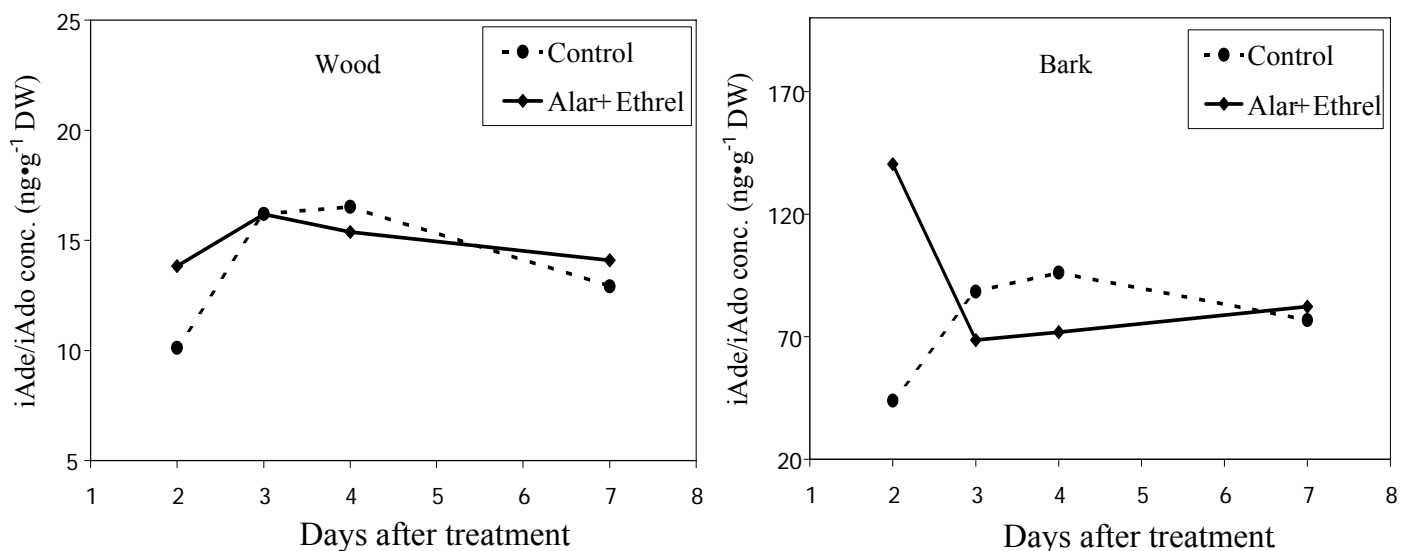


Figure 4.44. iAde/iAdo concentrations in wood and bark of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

## 4.2.2.2 Gibberellins

In growing shoots of cv. Golden Delicious the GA concentrations decreased after spraying with Alar plus Ethrel, while there was little difference between control and treatment in cv. Idared (Figure 4.45). Interestingly the GA concentration in wood and bark of cv. Golden Delicious was also reduced after spraying (Figure 4.46).

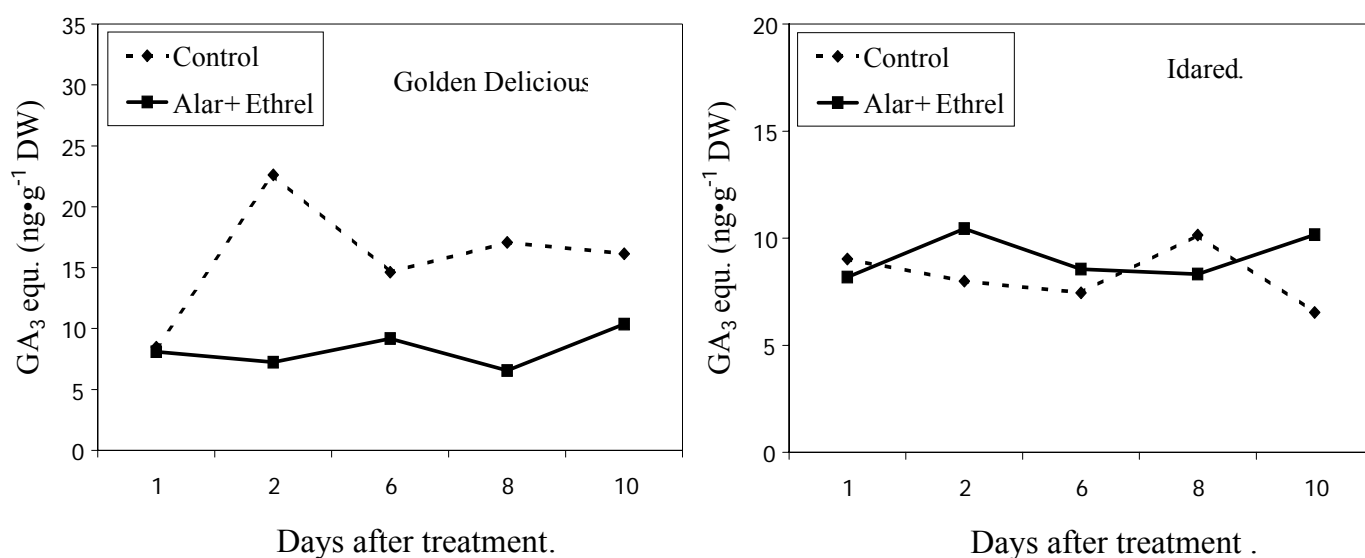


Figure 4.45. GA concentrations in growing shoots of apple trees of cv. Golden Delicious and Idared after spraying with Alar plus Ethrel

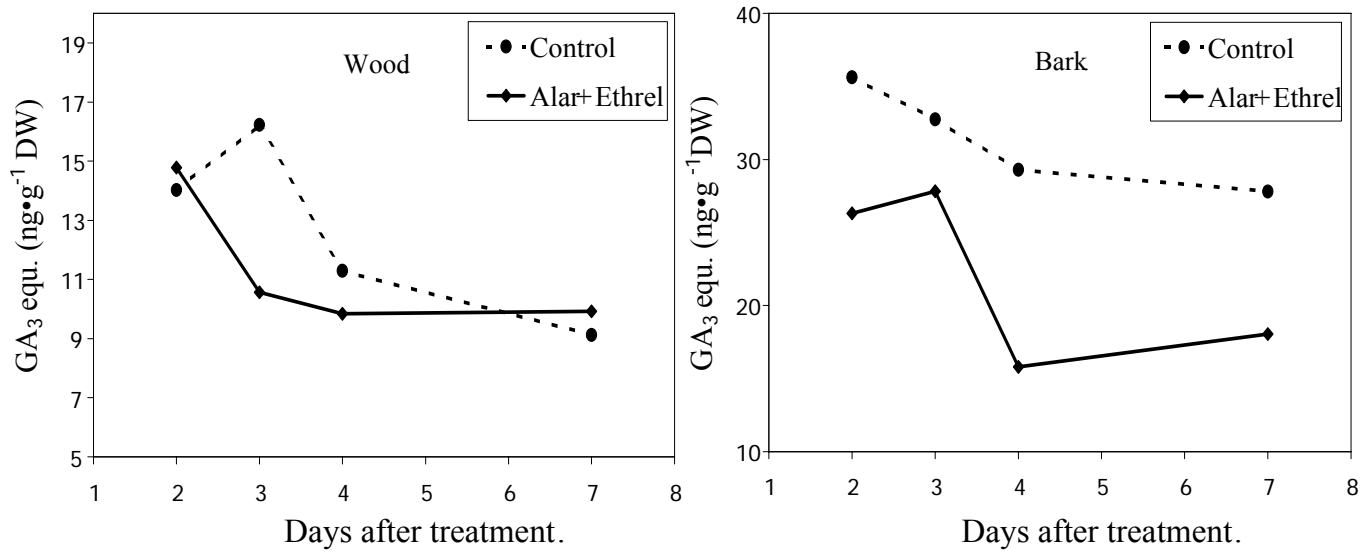


Figure 4.46. GA concentrations in wood and bark of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

#### 4.2.2.3 IAA

The concentrations of IAA in growing shoots were reduced after spraying with Alar plus Ethrel more consistently in Idared than in Golden Delicious (Figure 4.47). Contrary to this the IAA concentration in bark was higher, while there was practically no difference in wood after spraying (Figure 4.48).

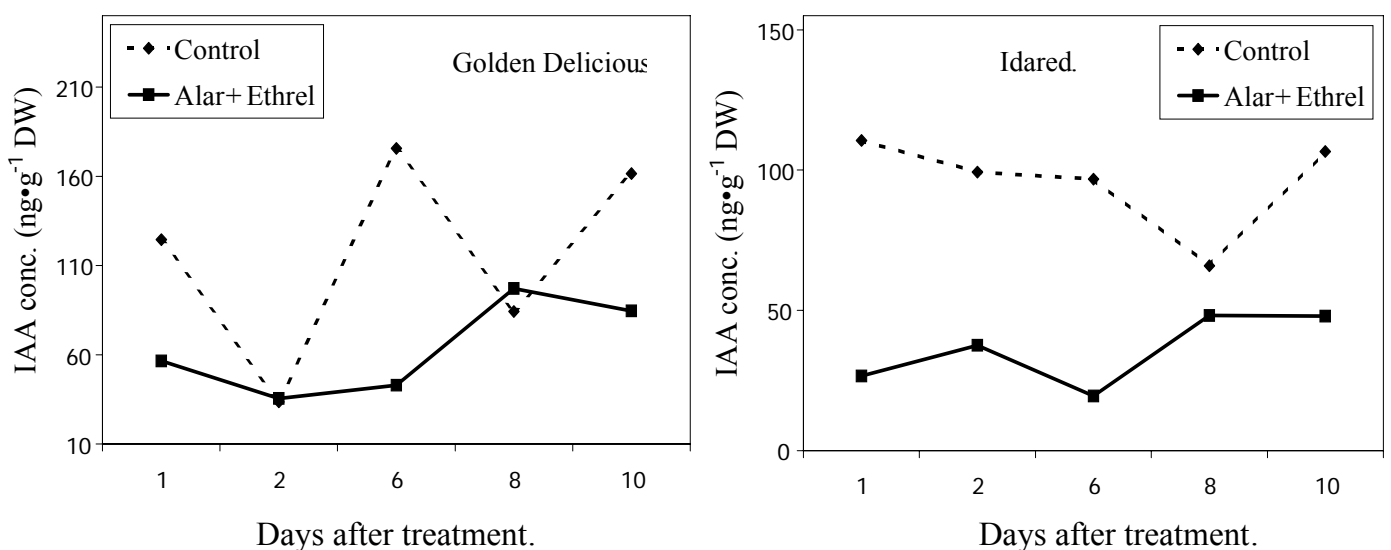


Figure 4.47. IAA concentrations in growing shoots of cv. Golden Delicious and Idared after spraying with Alar plus Ethrel

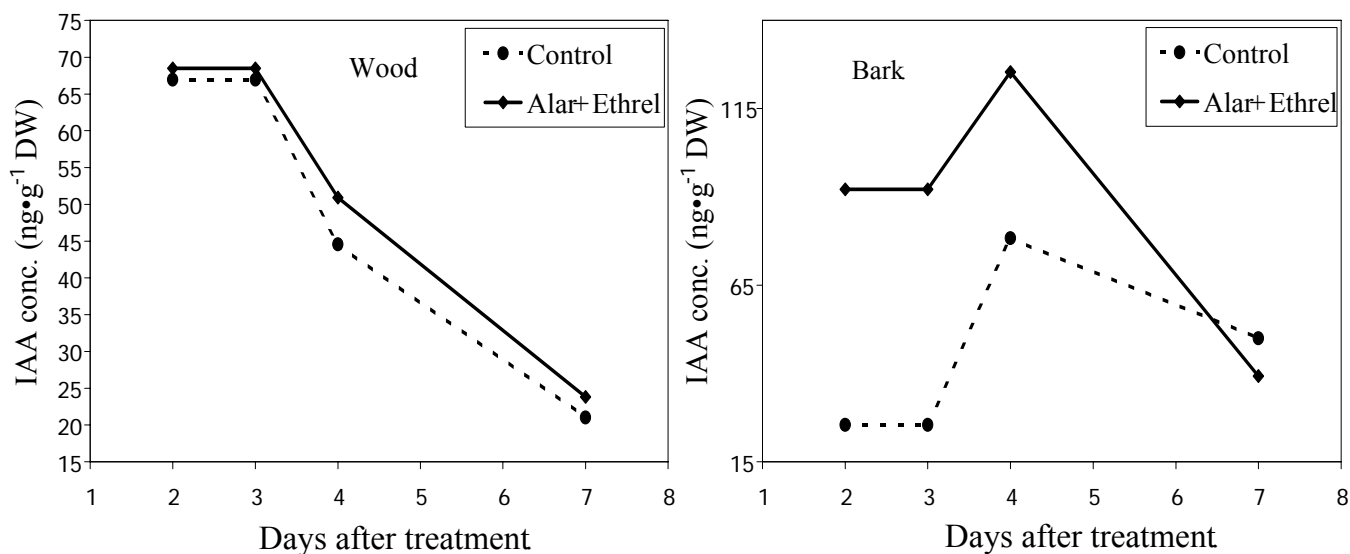


Figure 4.48. IAA concentrations in wood and bark of cv. Golden Delicious after spraying with Alar plus Ethrel

Interestingly, spraying with Alar plus Ethrel decreased significantly IAA concentrations in shoot exudates of cv. Golden Delicious when compared to control (Figure 4.49).

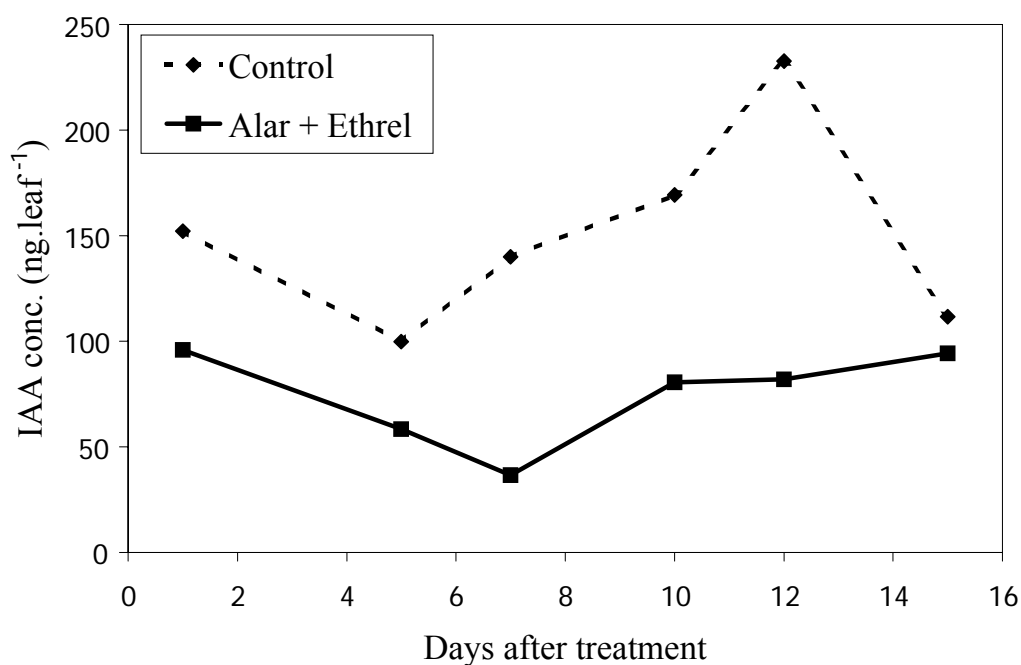


Figure 4.49. IAA concentrations in shoot exudates of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

#### 4.2.2.4 ABA

In both cultivars there were no clear tendencies of ABA concentrations in growing shoots between control and treatment (Figure 4.50).

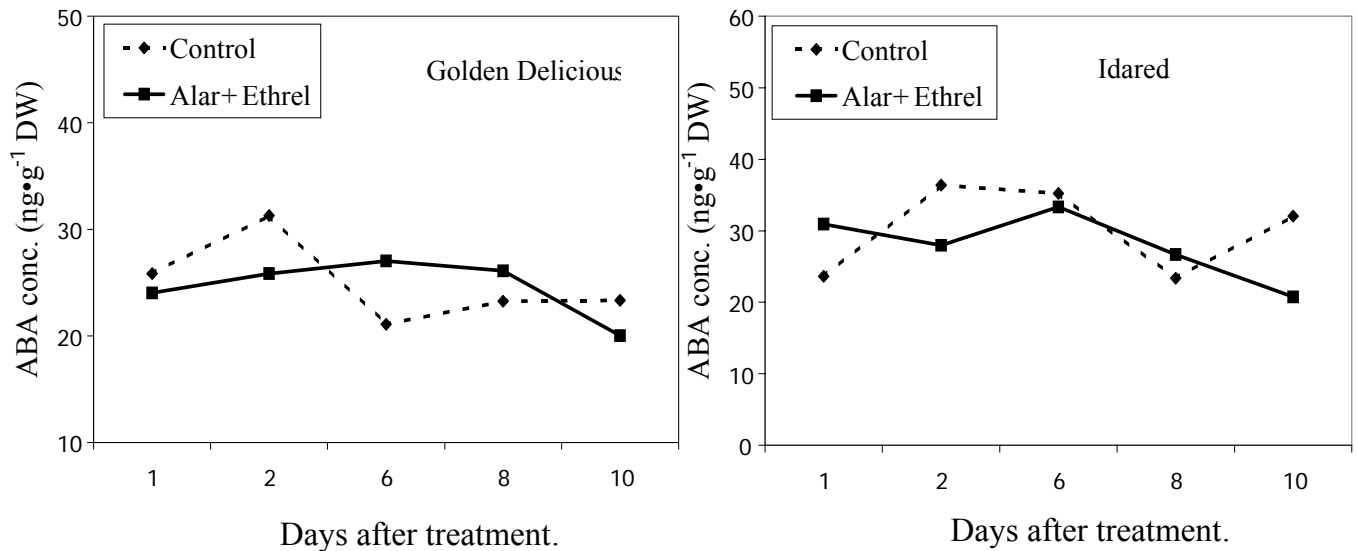


Figure 4.50. ABA concentrations in growing shoots of cv. Golden Delicious and Idared after spraying with Alar plus Ethrel

#### 4.2.2.5 Ethylene

Spraying Alar and Ethrel treatment increased the ethylene concentrations in Golden Delicious shoots during one week after treatment. After that, however, small differences were found at the second week (Figure 4.51)

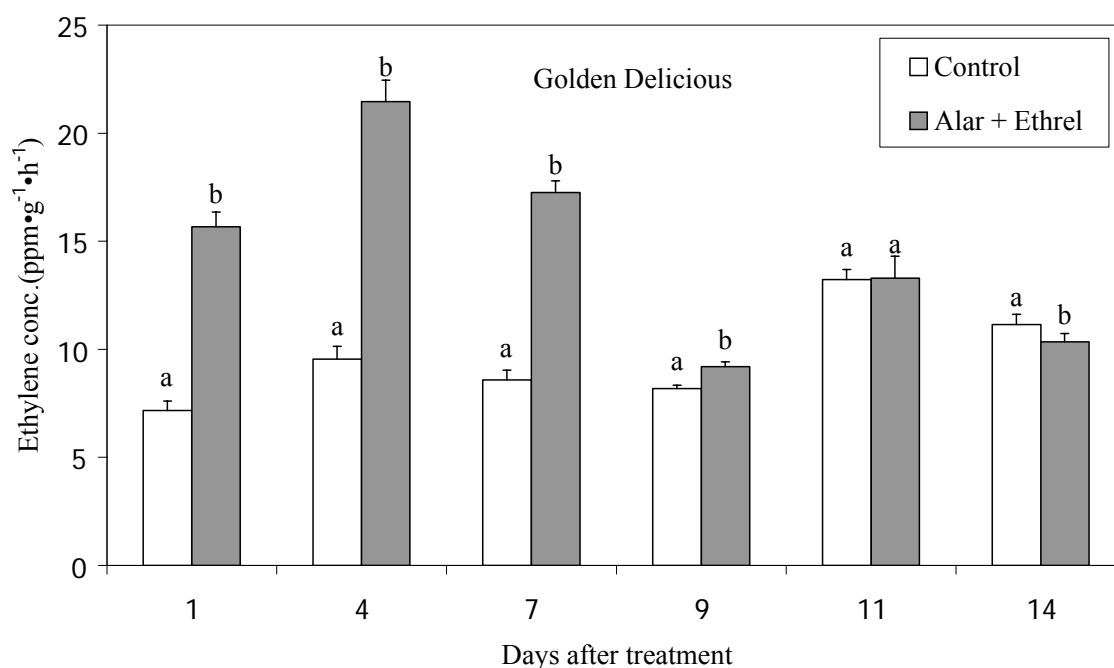


Figure 4.51. Ethylene concentrations in apple shoot of cv. Golden Delicious after spraying with Alar plus Ethrel

### 4.3 Experiments in 2003

#### 4.3.1 Effect of Alar and Ethrel on changes in hormone, starch and sugar contents in shoot tips and leaves of apple trees

##### 4.3.1.1 Cytokinins

Z/ZR concentrations in growing shoots increased in both cvs. Golden Delicious and Elstar after spraying with Alar plus Ethrel. (Figure 4.52, 4.53). These results were similar to Z/ZR concentrations in leaves of cv. Elstar (Figure 4.55) but not in cv. Golden Delicious (Figure 4.54). In non-growing shoots, increasing in Z/ZR concentrations was with one exception, not observed in both cultivars (Figure 4.52, 4.53). A consistent effect of Alar plus Ethrel treatments on Z/ZR concentration in leaves for both cvs. could not be observed (Figure 4.54, 4.55).



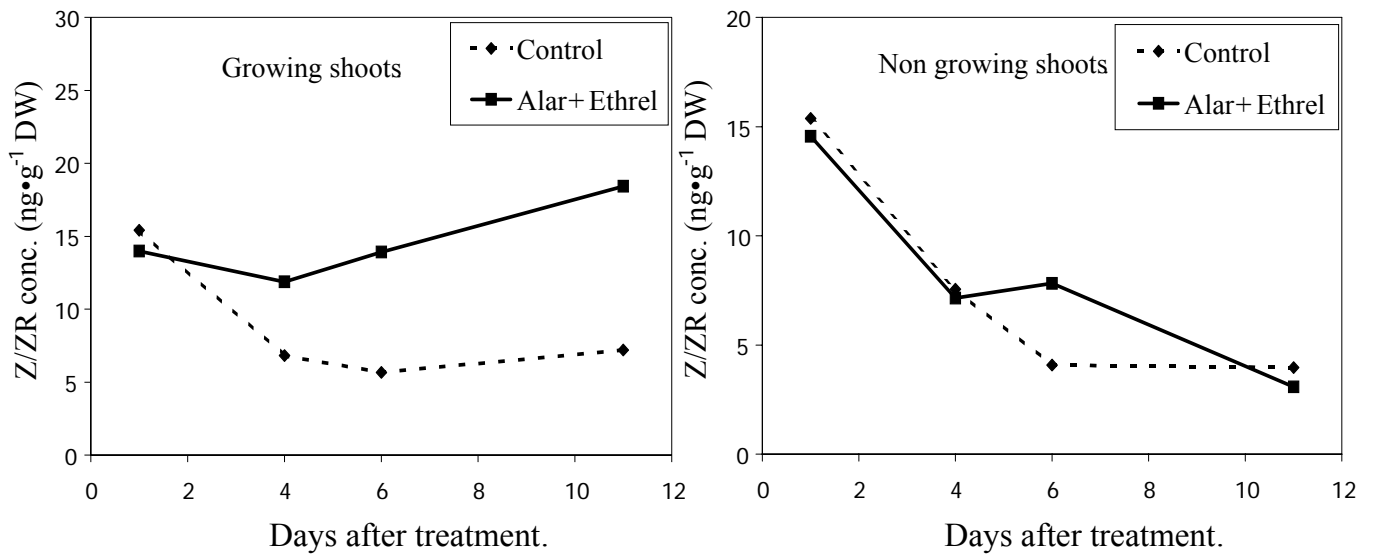


Figure 4.52. Z/ZR concentrations in growing and non-growing shoot tips of cv. Golden Delicious after spraying with Alar plus Ethrel

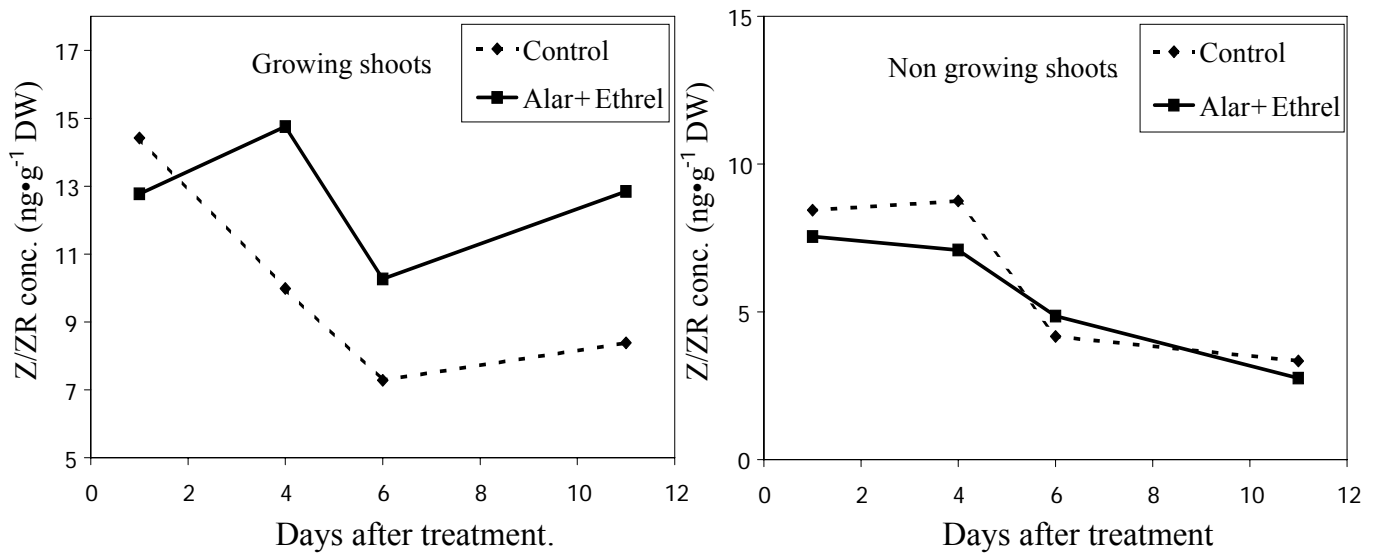


Figure 4.53. Z/ZR concentrations in growing and non-growing shoot tips of cv. Elstar after spraying with Alar plus Ethrel

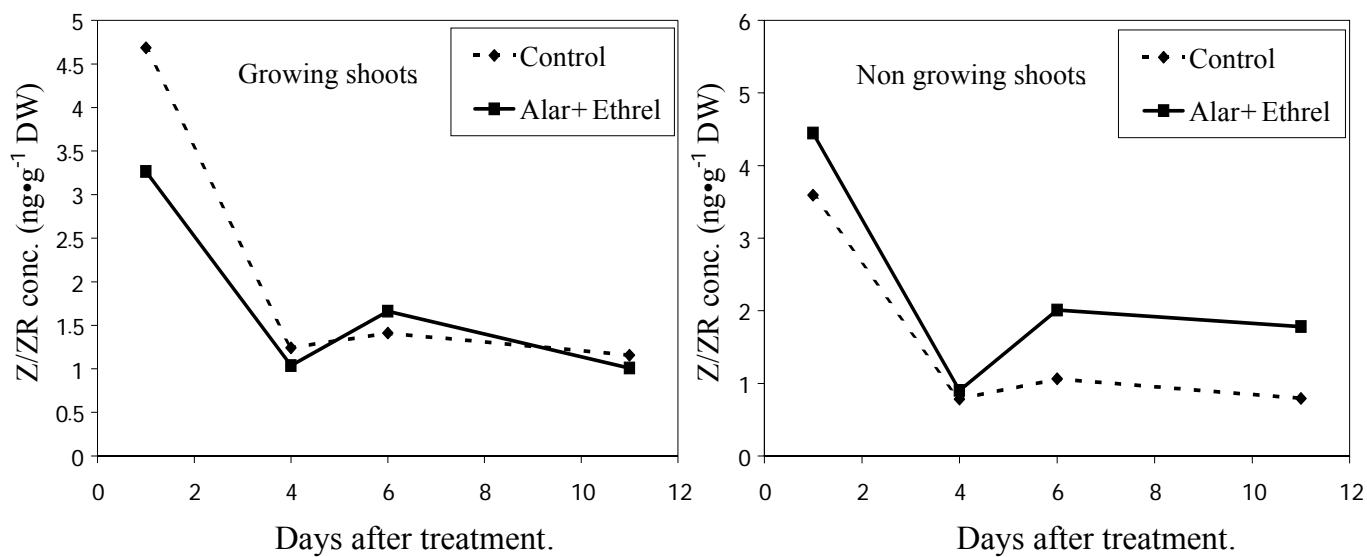


Figure 4.54. Z/ZR concentrations in leaves from growing and non-growing shoots of cv. Golden Delicious after spraying with Alar plus Ethrel

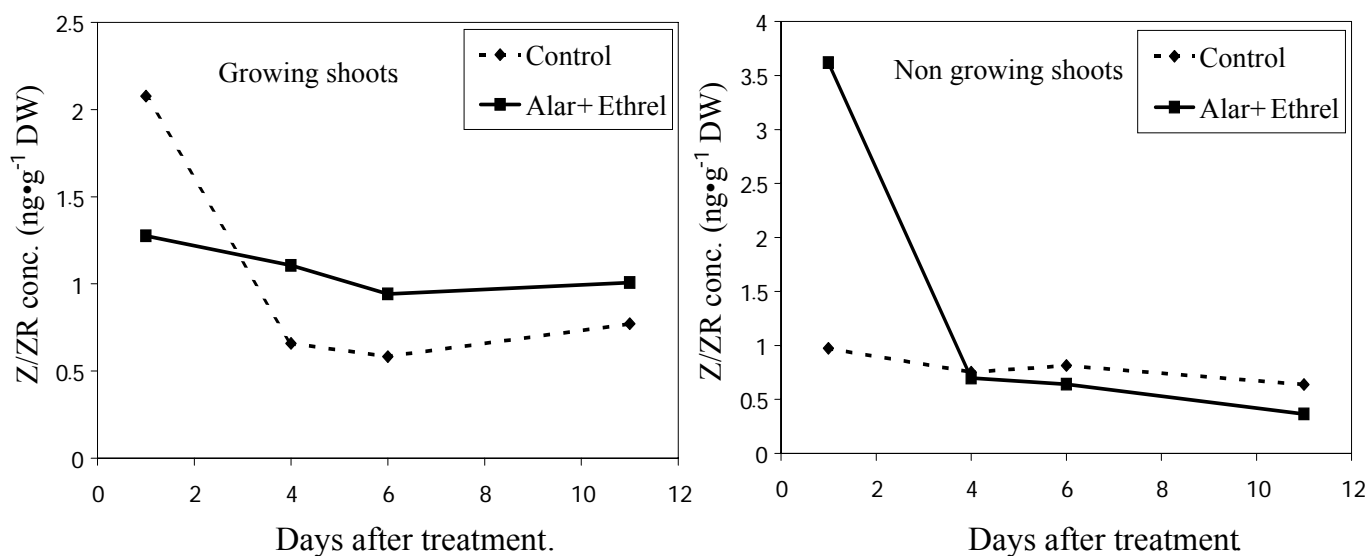


Figure 4.55. Z/ZR concentrations in leaves from growing and non-growing shoots of cv. Elstar after spraying with Alar plus Ethrel

## 4.3.1.2 Gibberellins

Contrary to Z/ZR, GA concentrations in growing shoots decreased in both cvs. Golden Delicious and Elstar after spraying Alar plus Ethrel when compared to control (Figure 4.56, 4.57). These results were not found in leaves, except only at 1 and 11 day after spraying in cv. Golden Delicious (Figure 4.58, 4.59). In non-growing shoots and also in their leaves, decreasing in GA concentrations could observe hardly in both cultivars after spraying (Figure 4.56- 4.59).

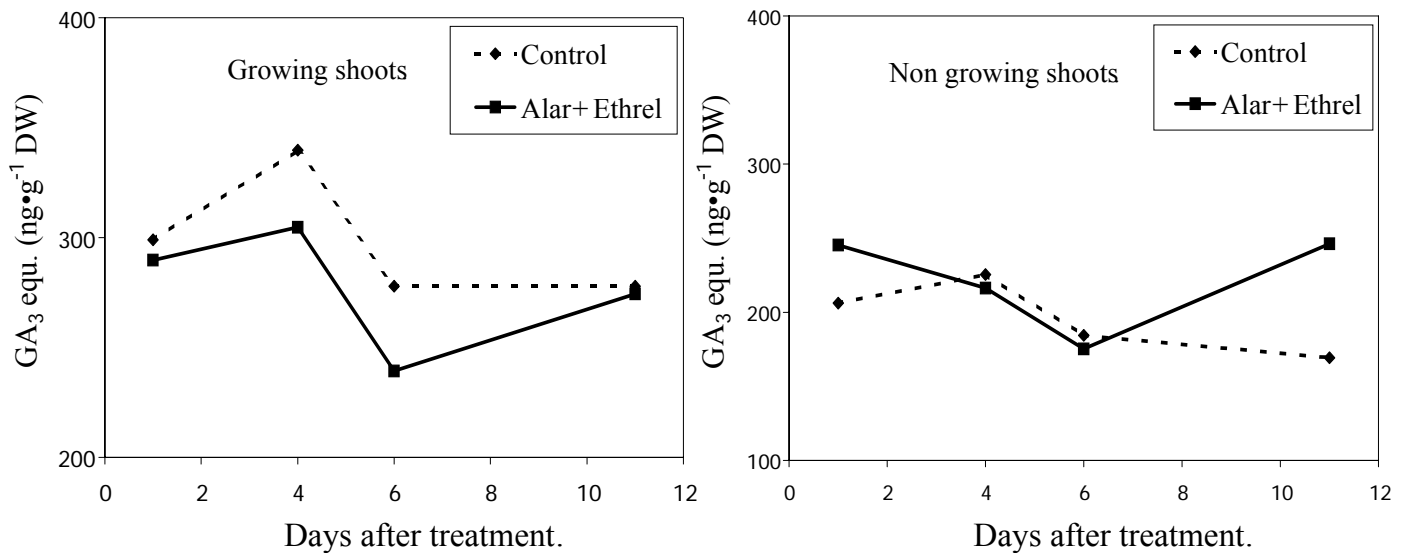


Figure 4.56. GA concentrations in growing and non-growing shoot of cv. Golden Delicious after spraying with Alar plus Ethrel

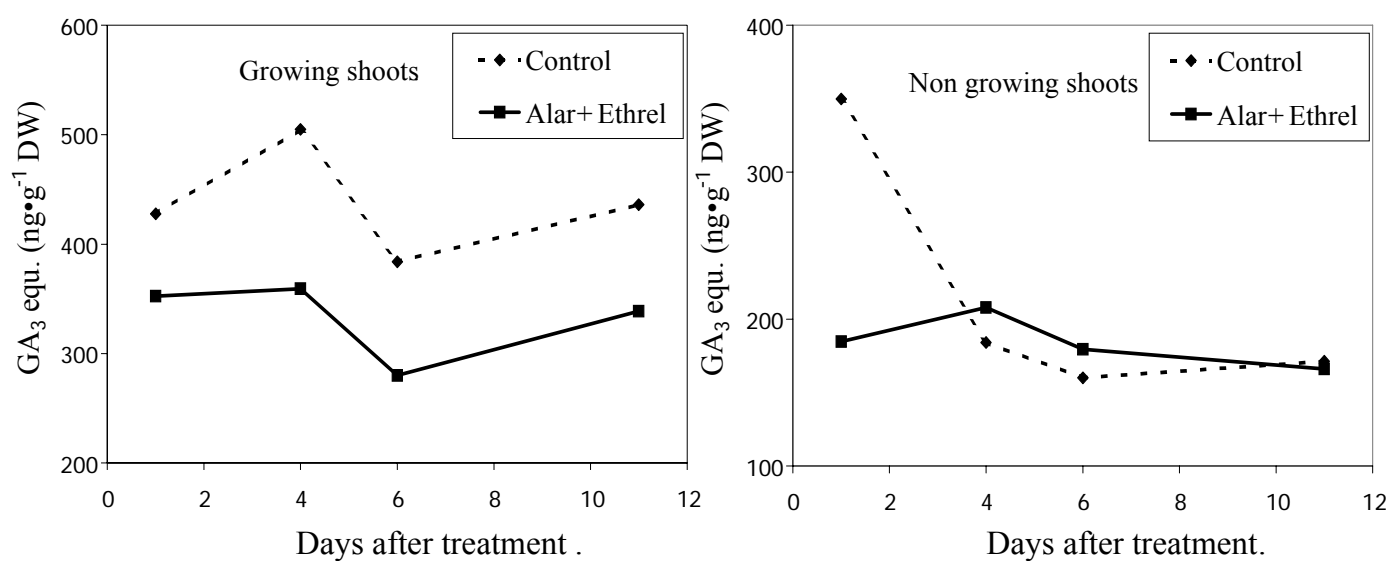


Figure 4.57. GA concentrations in growing and non-growing shoot of cv. Elstar after spraying with Alar plus Ethrel

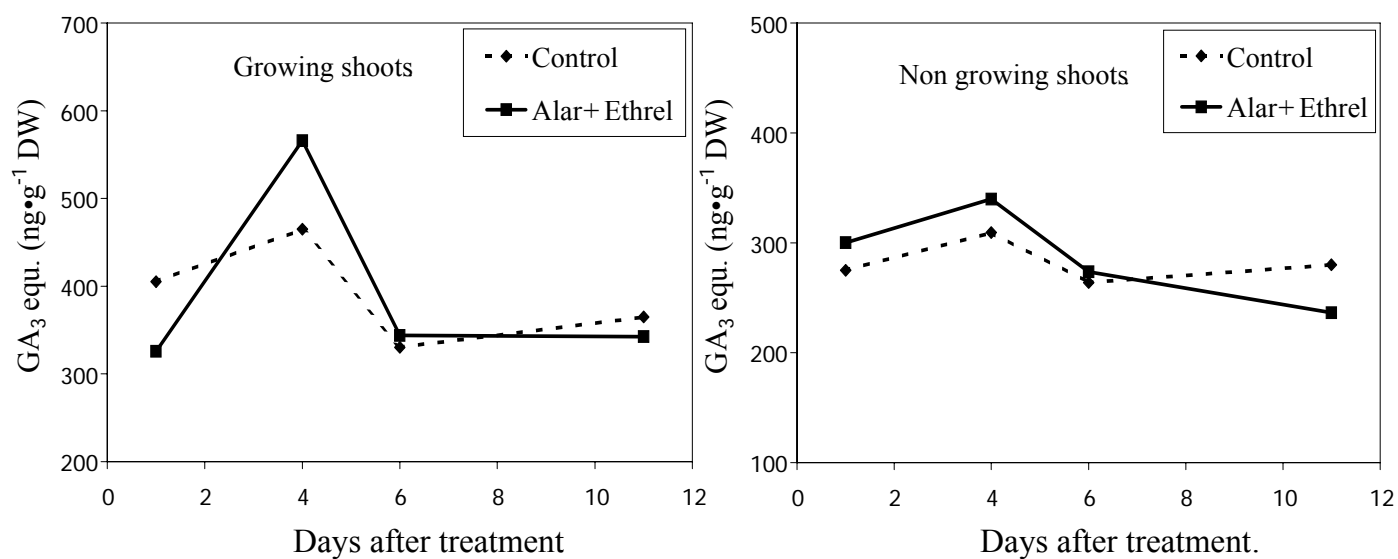


Figure 4.58. GA concentrations in leaves from growing and non-growing shoots of cv. Golden Delicious after spraying with Alar plus Ethrel

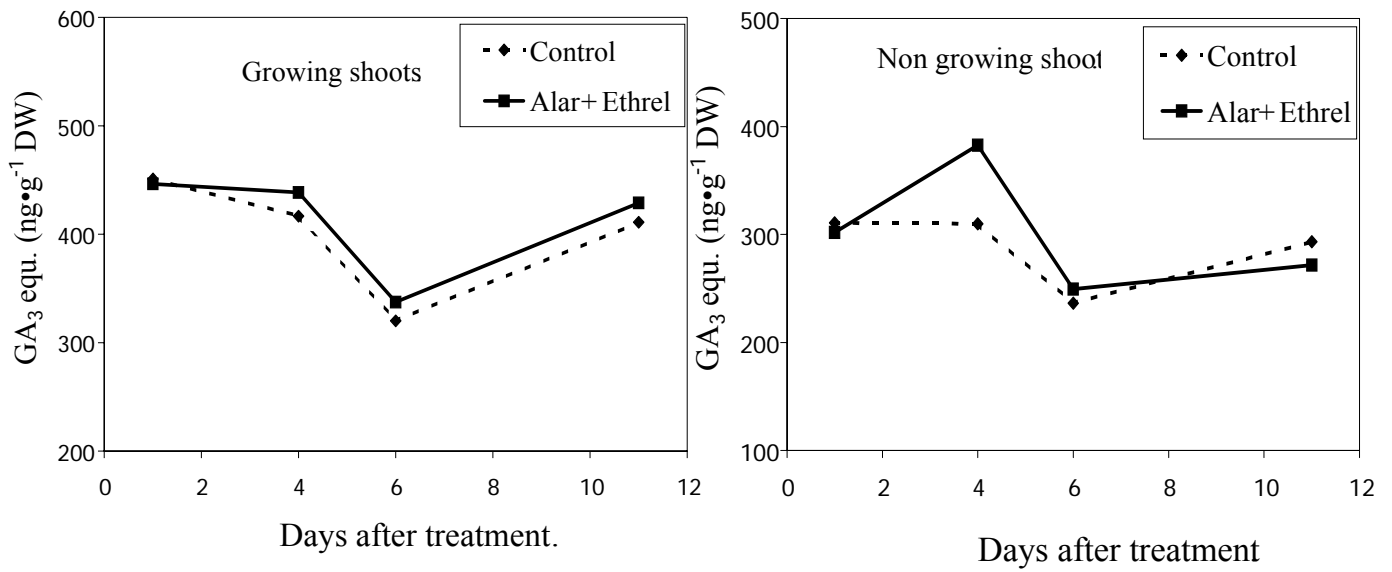


Figure 4.59. GA concentrations in leaves from growing and non-growing shoots of cv. Elstar after spraying with Alar plus Ethrel

#### 4.3.1.3 IAA

Similar to GAs, IAA concentrations in growing shoots decreased in both cvs. Golden Delicious and Elstar after spraying Alar plus Ethrel (Figure 4.60, 4.61). In their leaves no consistent effects were observed (Figure 4.62, 4.63). In non-growing shoots and their leaves, no clear tendency of changing values could be detected (Figure 4.60-4.63).

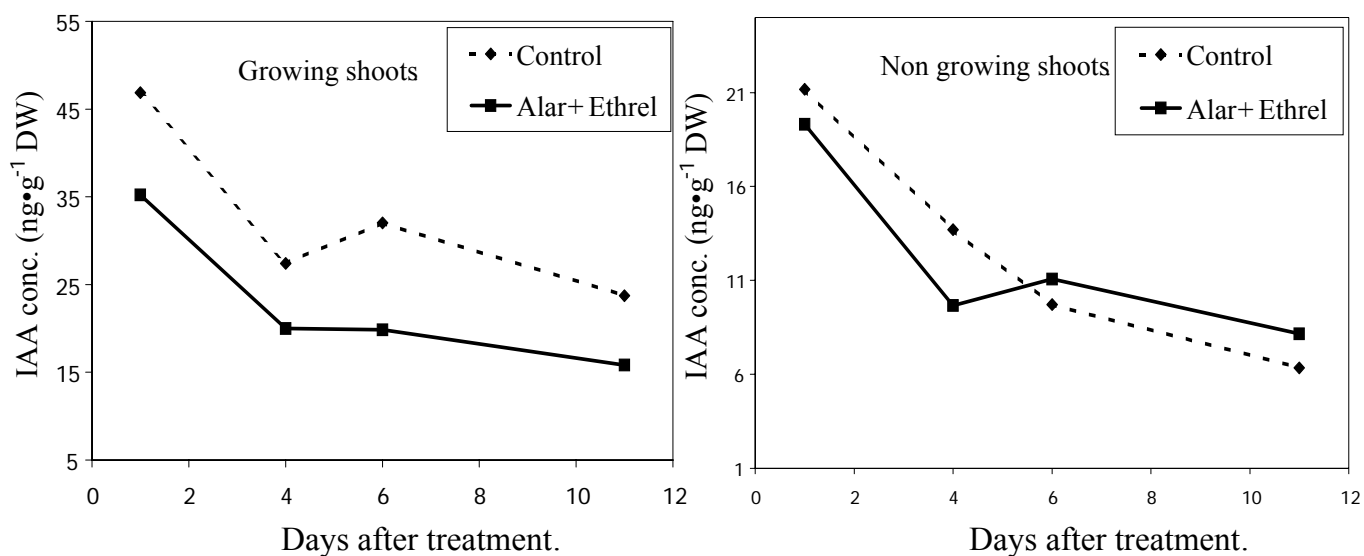


Figure 4.60. IAA concentrations in growing and non-growing shoots of cv. Golden Delicious after spraying with Alar plus Ethrel

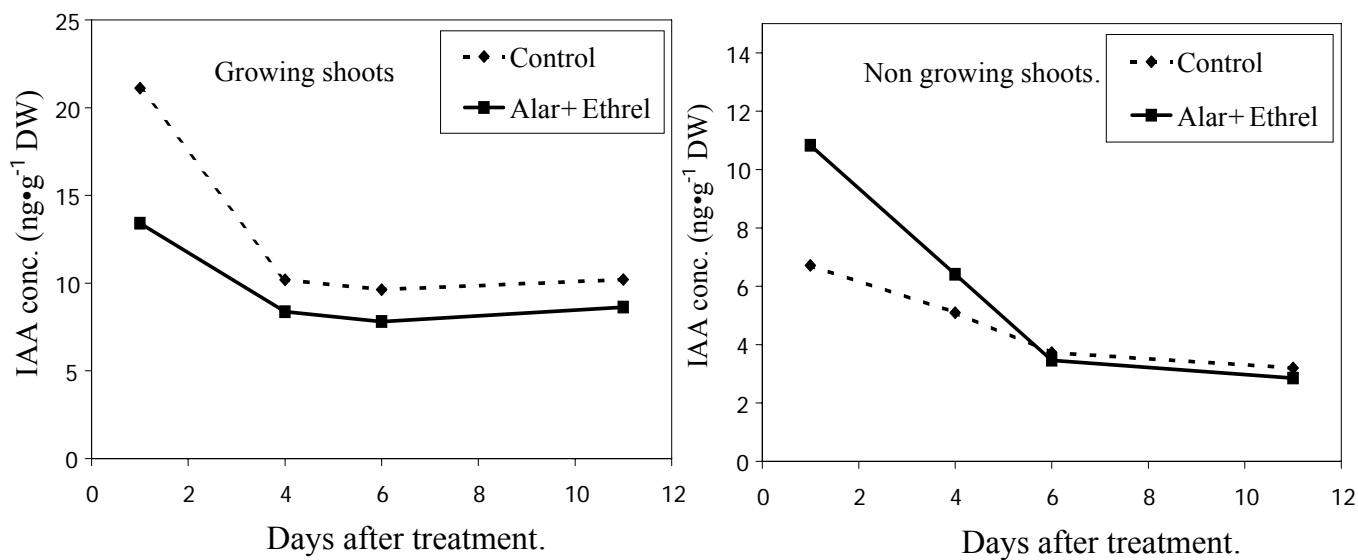


Figure 4.61. IAA concentrations in growing and non-growing shoots of cv. Elstar after spraying with Alar plus Ethrel

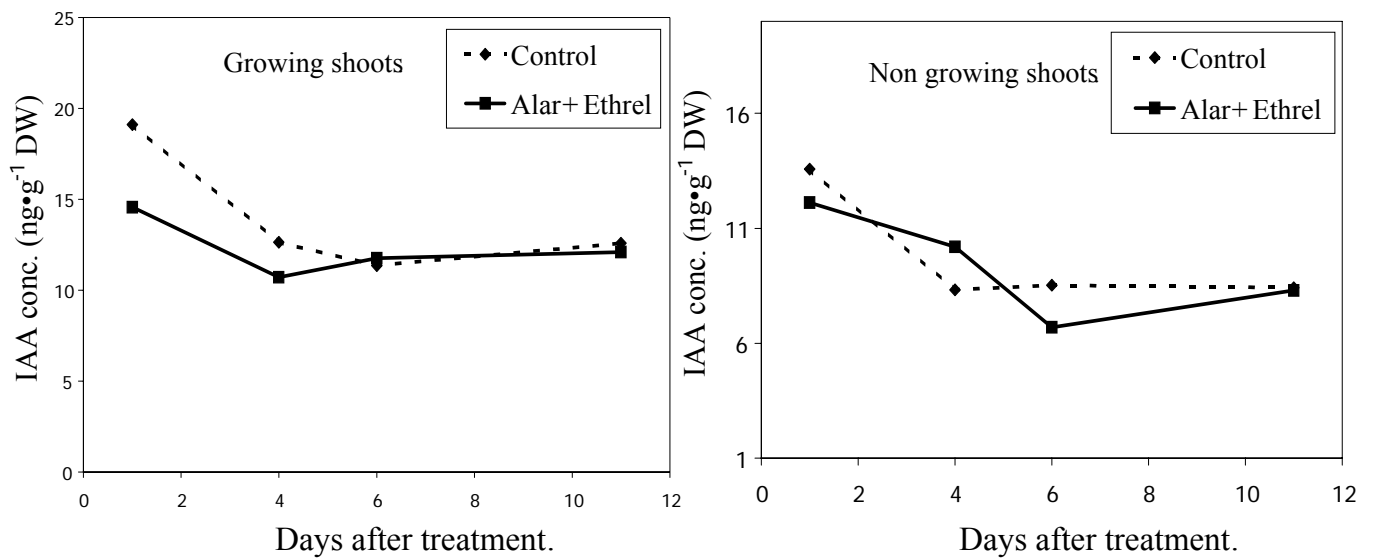


Figure 4.62. IAA concentrations in leaves from growing and non-growing shoots of cv. Golden Delicious after spraying with Alar plus Ethrel

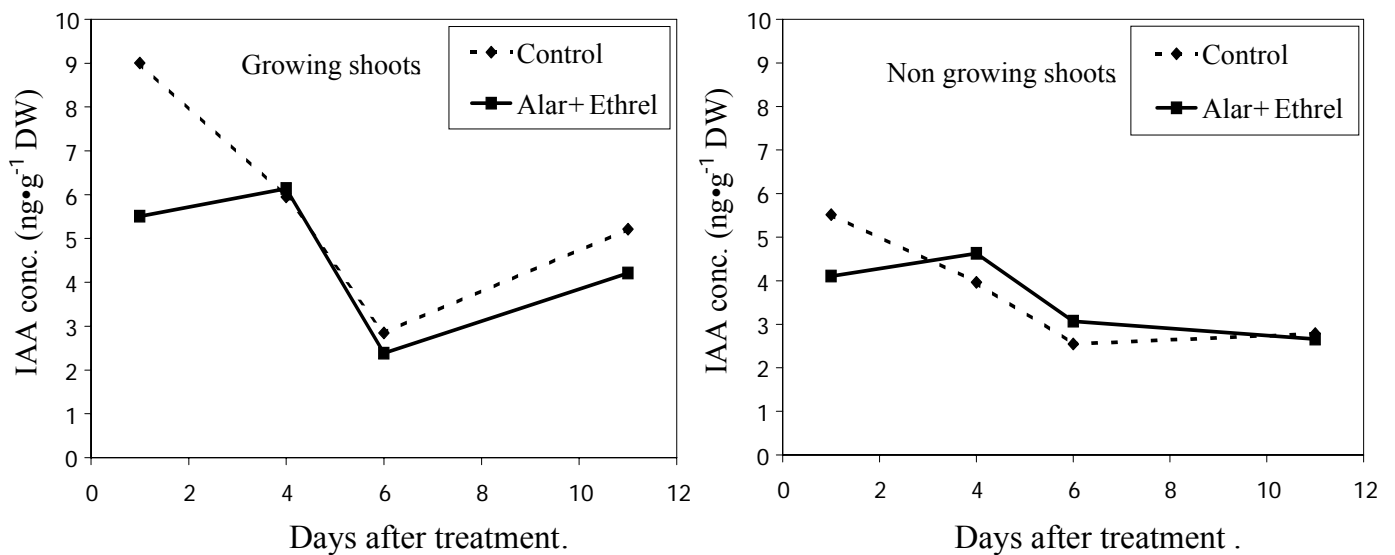


Figure 4.63. IAA concentrations in leaves from growing and non-growing shoots of cv. Elstar after spraying with Alar plus Ethrel

## 4.3.1.4 Ethylene

Ethylene concentrations increased significantly in both cvs. Golden Delicious and Elstar after spraying with Alar and Ethrel (Figure 4.64, 4.65).

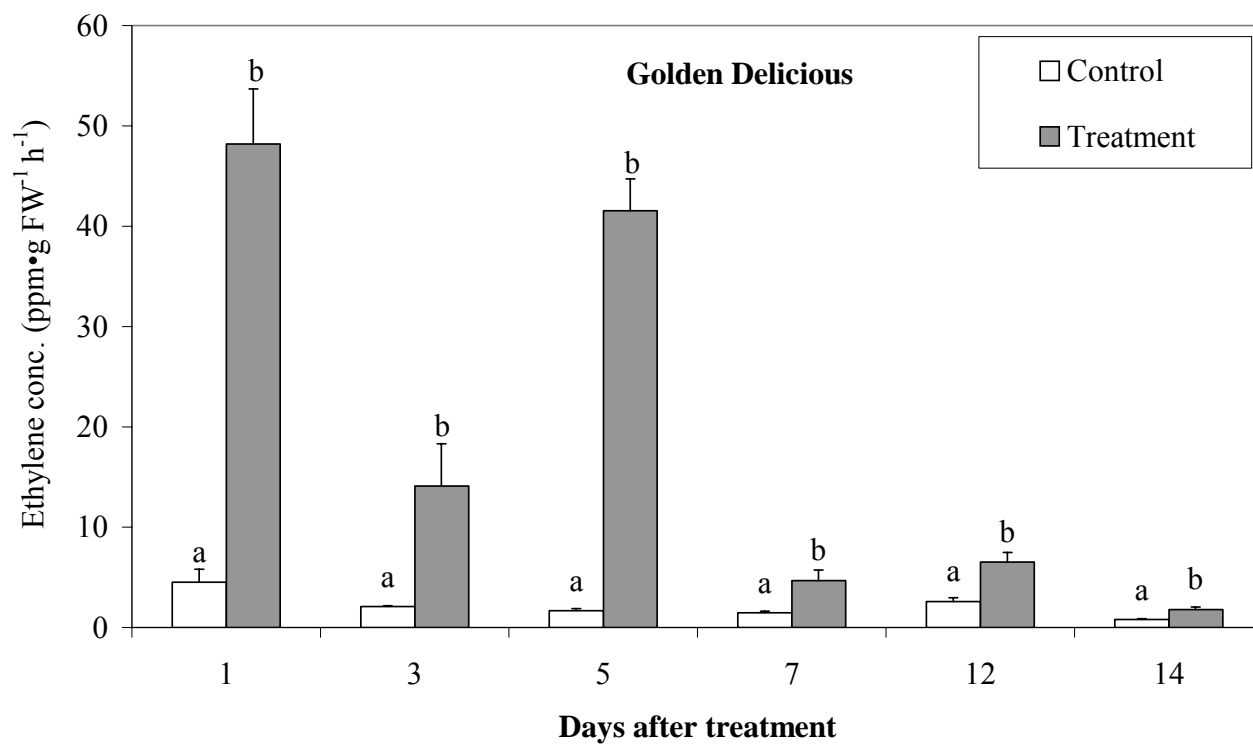


Figure 4.64. Ethylene concentrations in shoot of cv. Golden Delicious after spraying with Alar plus Ethrel



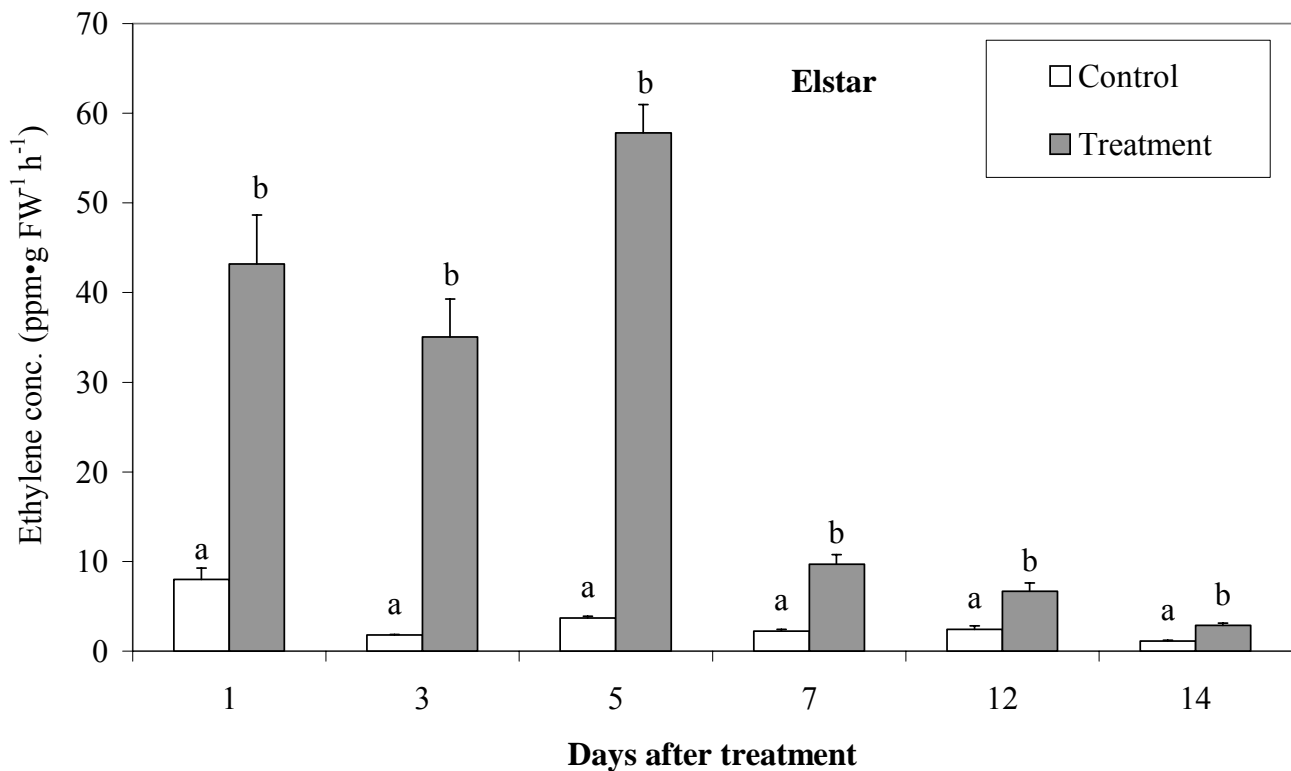


Figure 4.65. Ethylene concentrations in shoot of Elstar after spraying with Alar plus Ethrel

#### 4.3.1.5 Starch and sugar concentrations

##### 4.3.1.5.1 Reducing sugar

Spraying with Alar and Ethrel seemed to decrease reducing-sugar concentrations in growing shoots and their leaves (Figure 4.66, 4.67 and 4.69), except in growing shoots of cv. Elstar where higher concentration could be observed after spraying (Figure 4.68). In non-growing shoots, spraying seemed to increase reducing-sugar concentrations but decreased them in the leaves (Figure 4.66-4.69).

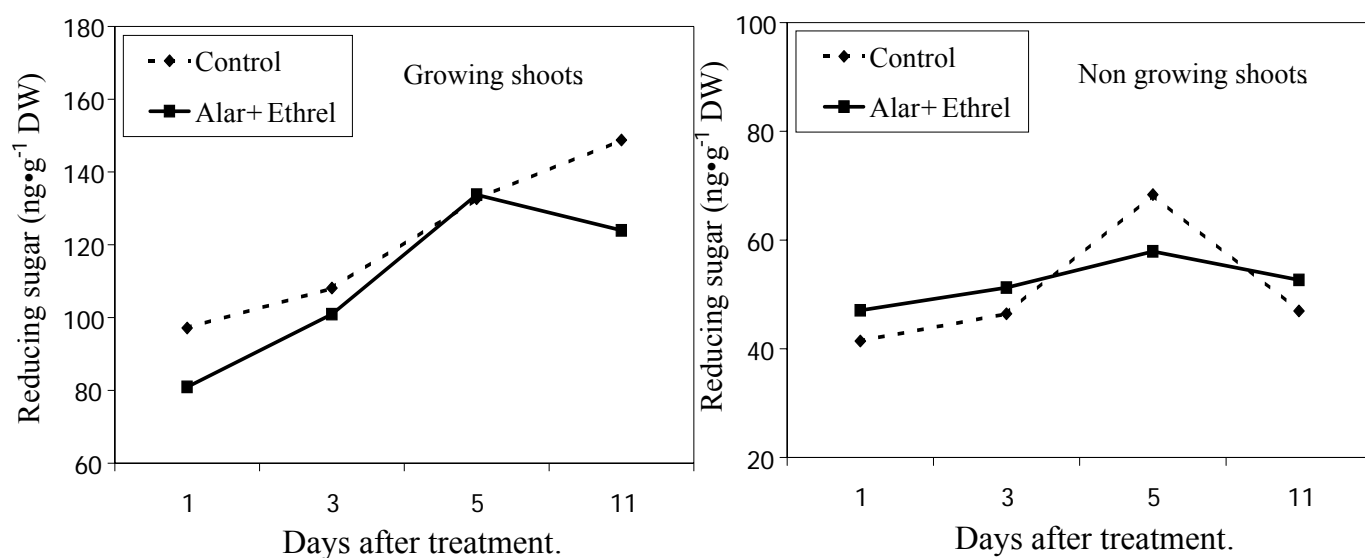


Figure 4.66. Reducing sugar concentrations in growing and non-growing shoots of cv. Golden Delicious after spraying with Alar plus Ethrel

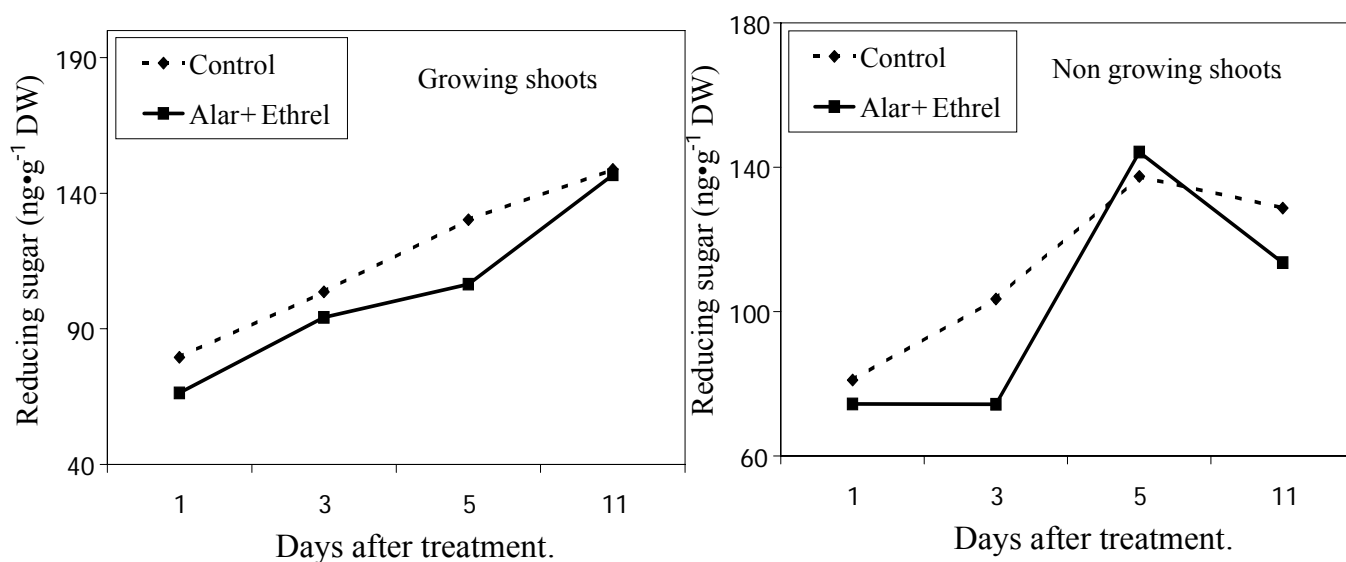


Figure 4.67. Reducing sugar concentrations in leaves from growing and non-growing shoots of cv. Golden Delicious after spraying with Alar plus Ethrel

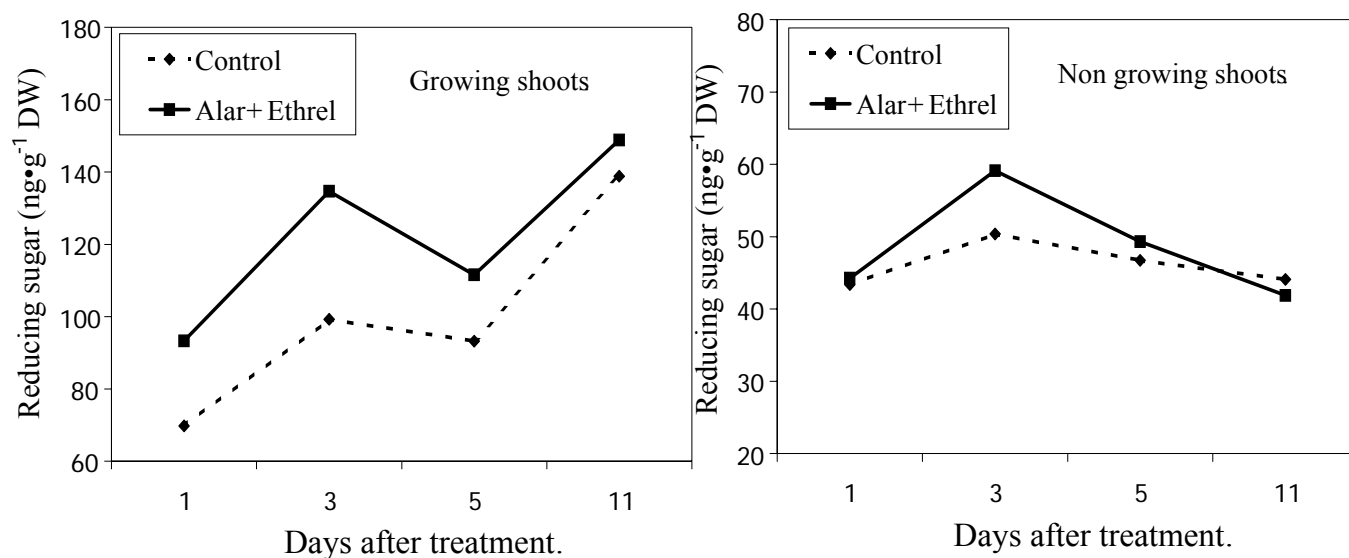


Figure 4.68. Reducing sugar concentrations in growing and non-growing shoots of cv. Elstar after spraying with Alar plus Ethrel

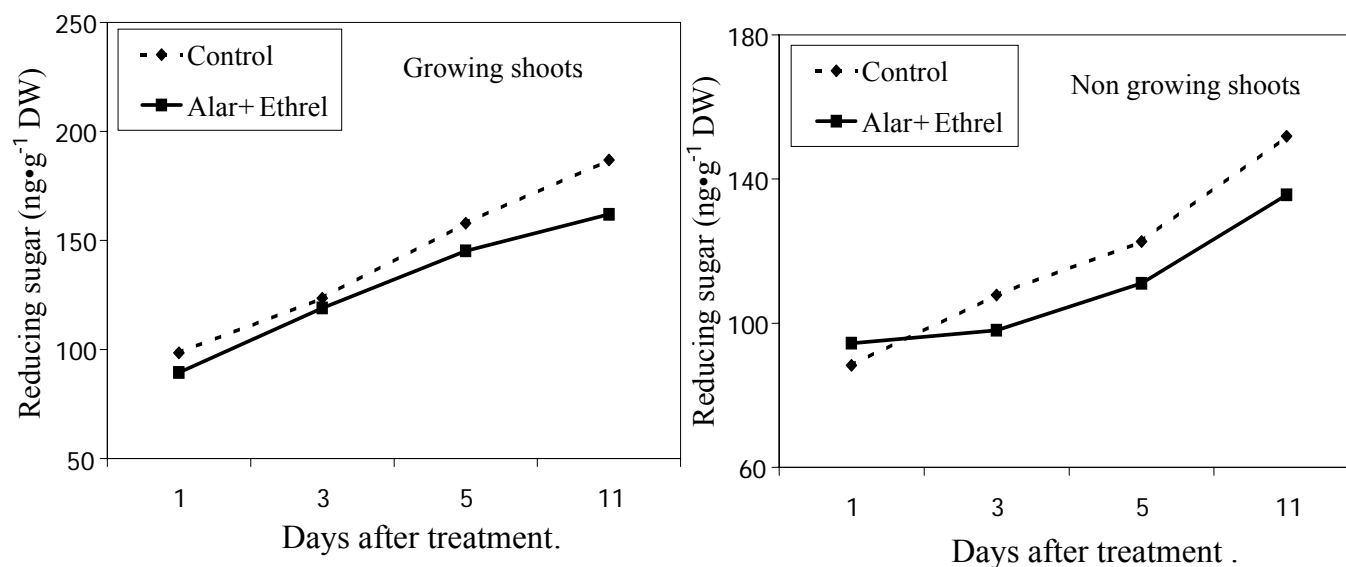


Figure 4.69. Reducing sugar concentrations in leaves from growing and non-growing shoots of cv. Elstar after spraying with Alar plus Ethrel

## 4.3.1.5.2 Sucrose concentrations

Sucrose concentrations in growing shoots were not different between control and treated trees of both cvs. Golden Delicious and Elstar (Figure 4.70 and 4.72) but in their leaves, spraying seemed to decrease sucrose concentrations in both cultivars (Figure 4.71 and 4.73). In non-growing shoots higher sucrose concentrations were observed in cv. Golden Delicious after spraying (Figure 4.70). However similar changes could only be observed in cv. Elstar only at 3 days after spraying (Figure 4.72). Similar to the leaves from growing shoots, spraying with Alar plus Ethrel also seemed to decrease sucrose concentrations in the leave from non-growing shoots in both cultivars (Figure 4.71 and 4.73).

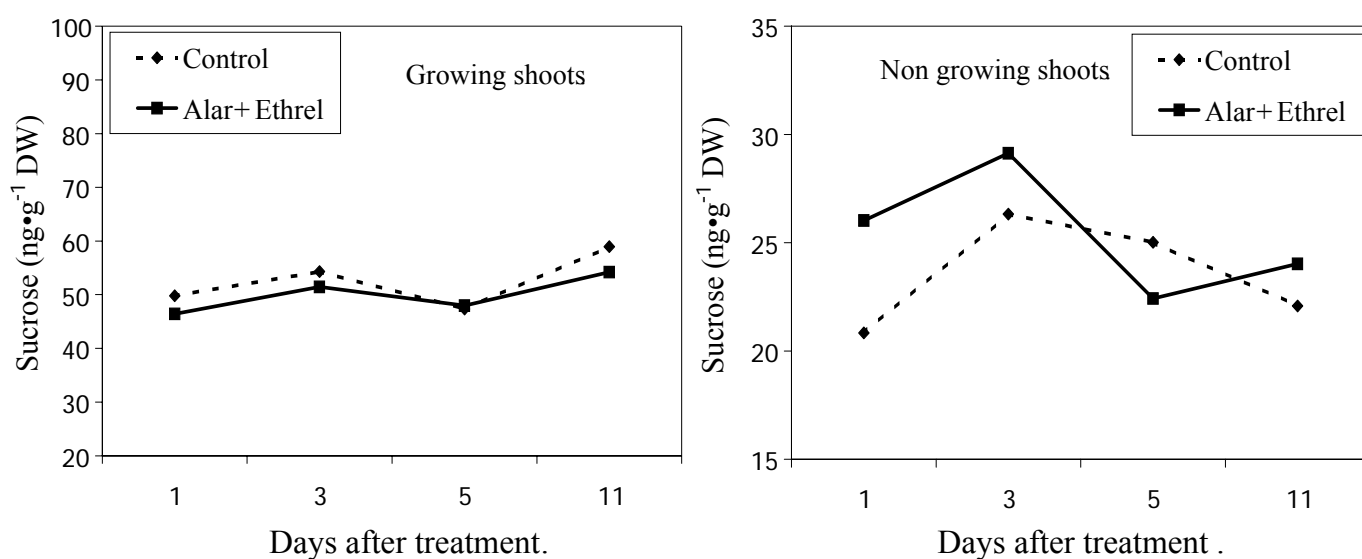


Figure 4.70. Sucrose concentrations in growing and non-growing shoots of cv. Golden Delicious after spraying with Alar plus Ethrel

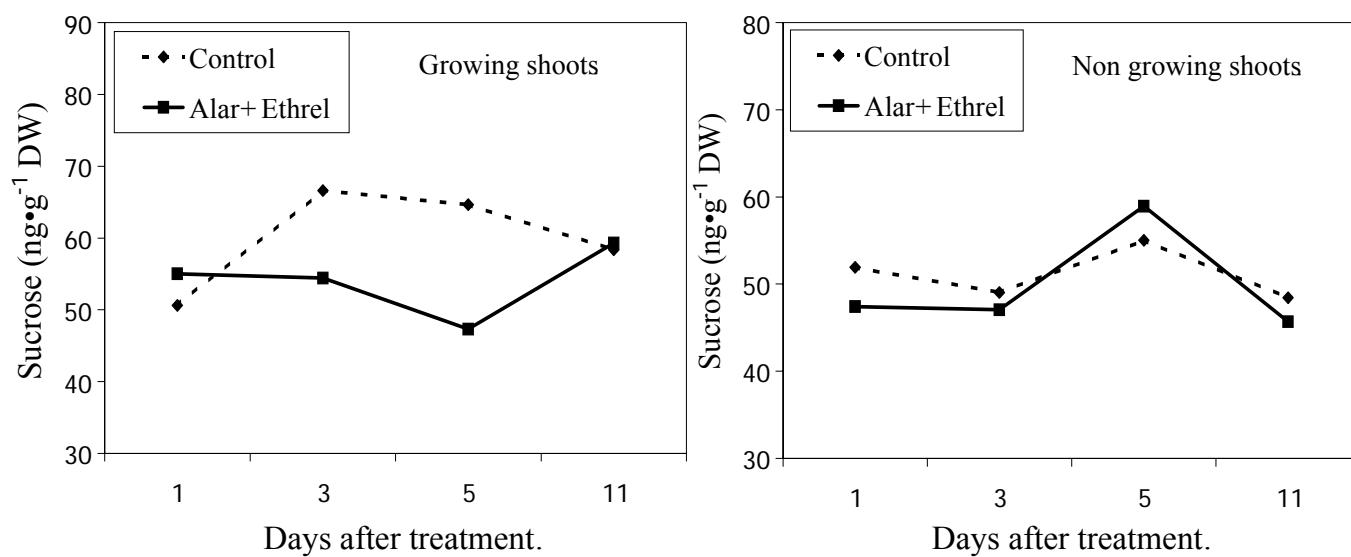


Figure 4.71. Sucrose concentrations in leaves from growing and non-growing shoots of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

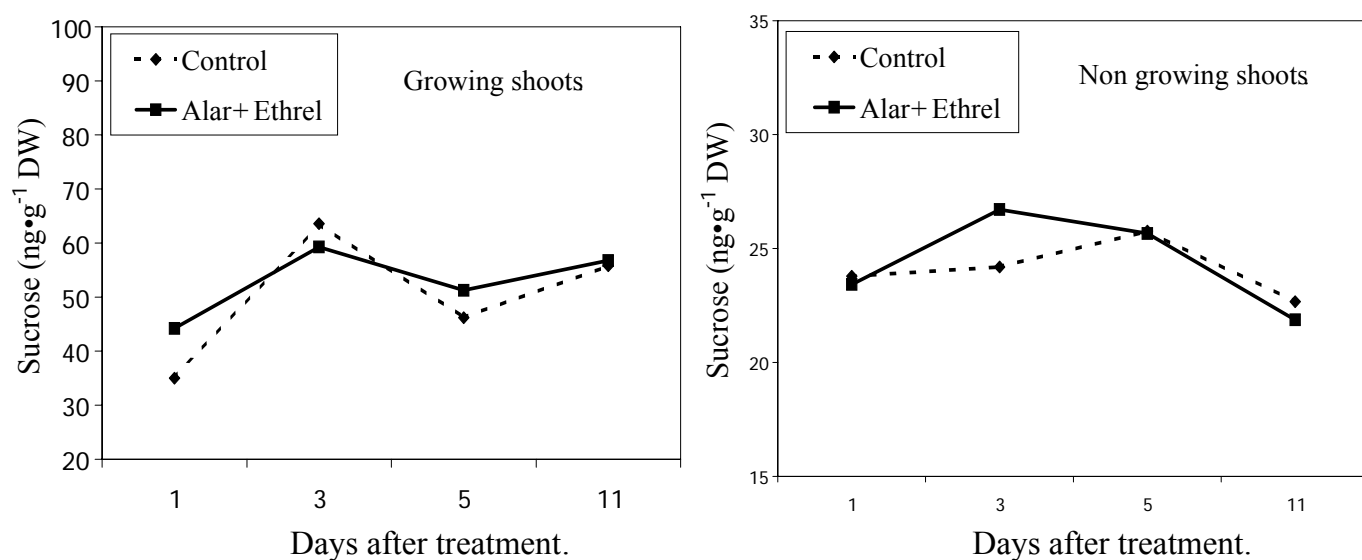


Figure 4.72. Sucrose concentrations in growing and non-growing shoots of apple trees of cv. Elstar after spraying with Alar plus Ethrel

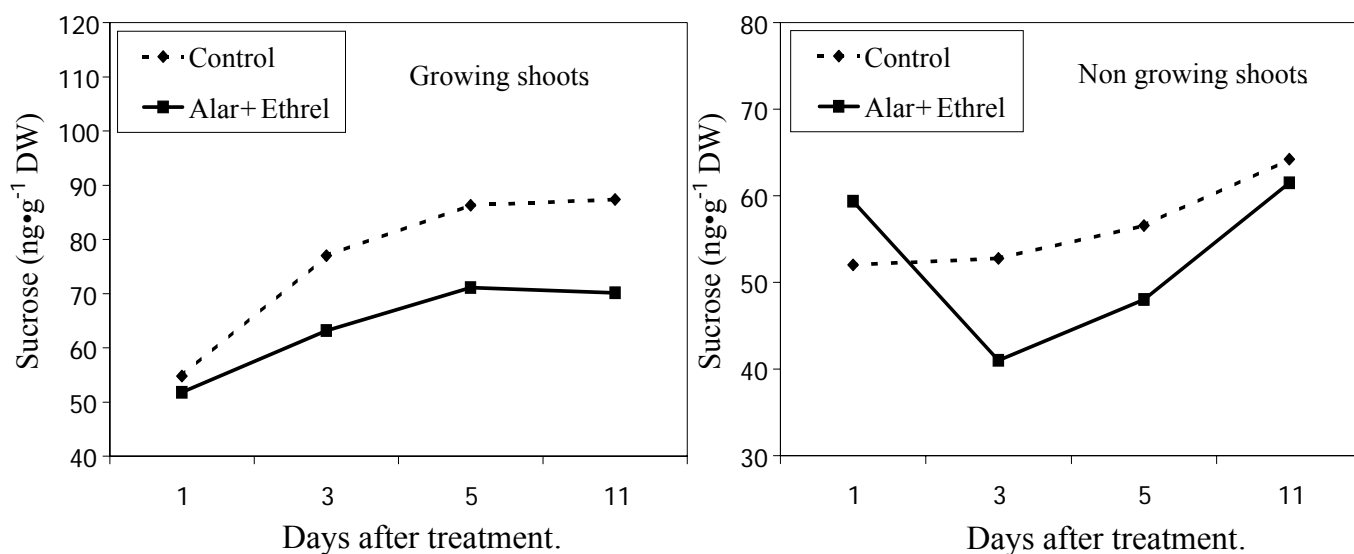


Figure 4.73. Sucrose concentrations in leaves from growing and non-growing shoots of apple trees of cv. Elstar after spraying with Alar plus Ethrel

#### 4.3.1.5.3 Starch concentrations

Spraying with Alar plus Ethrel seemed to decrease starch concentrations in growing shoots and leaves in cv. Golden Delicious but there were no clear tendencies in cv. Elstar. In non-growing shoots and their leaves, decreasing in starch concentrations also could be observed except in the leaves of cv. Elstar that showed higher starch concentration after spraying (Figure 4.74-4.77).

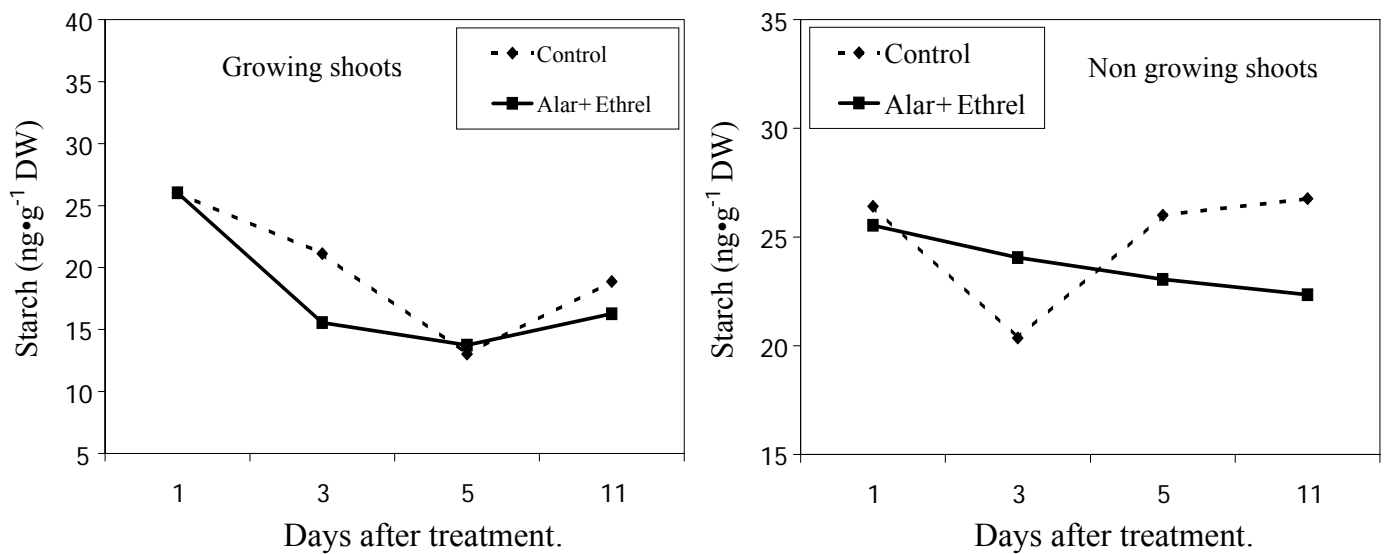


Figure 4.74. Starch concentrations in growing and non-growing shoots of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

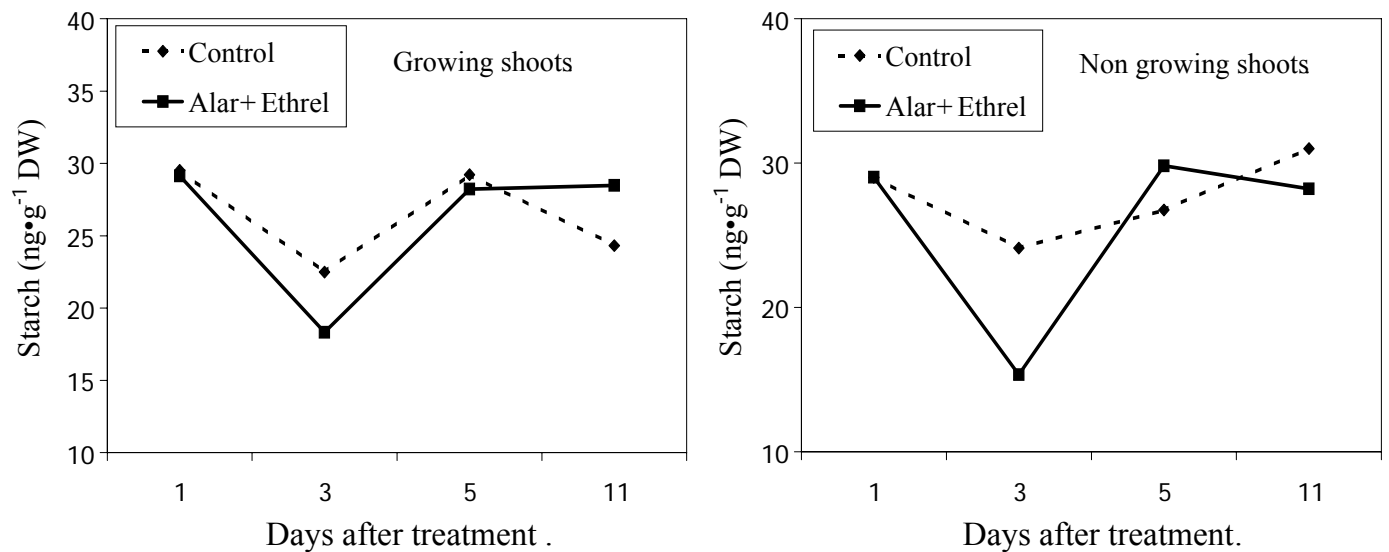


Figure 4.75 Starch concentrations in leaves from growing shoots and non-growing shoots of apple trees of cv. Golden Delicious after spraying with Alar plus Ethrel

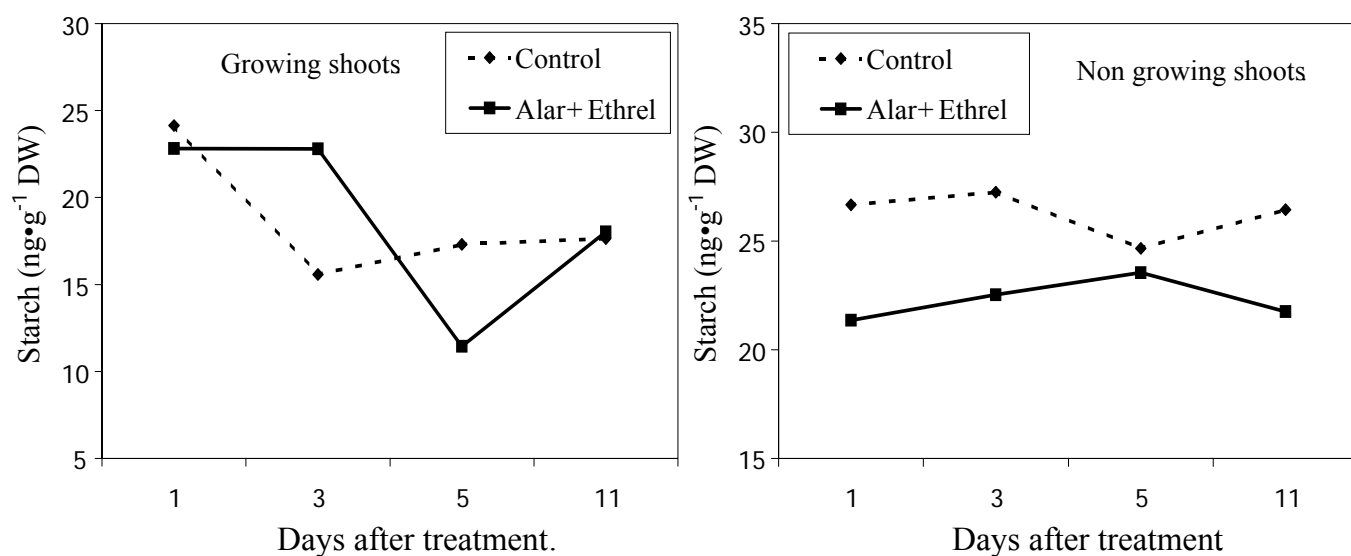


Figure 4.76. Starch concentrations in growing and non-growing shoots of apple trees of cv. Elstar after spraying with Alar plus Ethrel

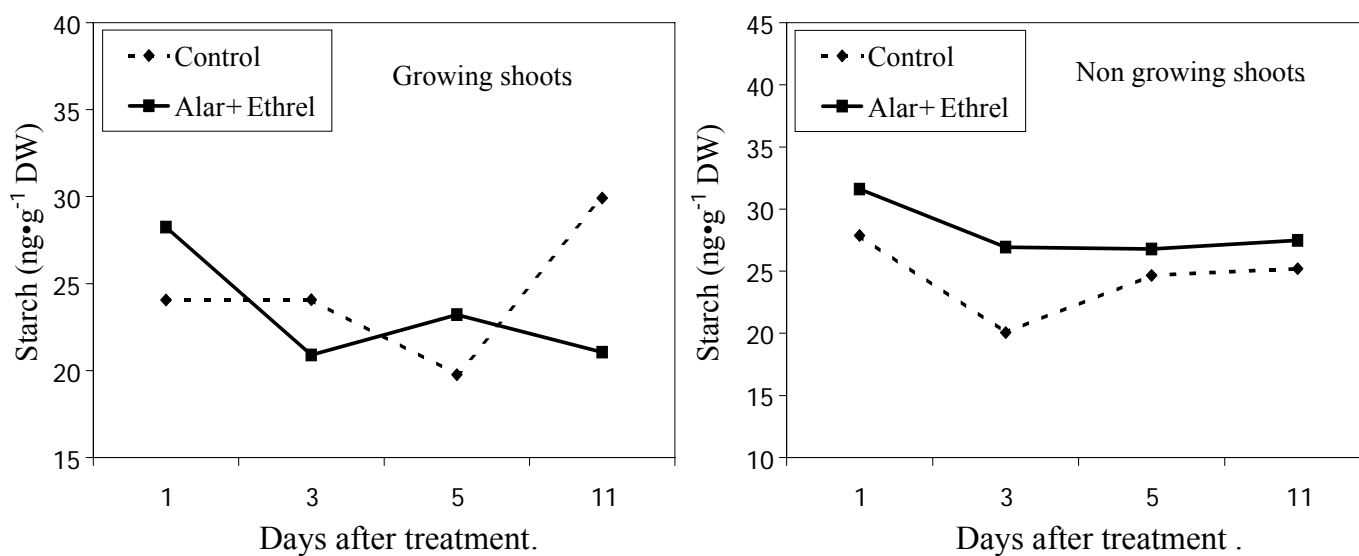


Figure 4.77. Starch concentrations in leaves from growing and non-growing shoots of apple trees of cv. Elstar after spraying with Alar plus Ethrel



## 5 DISCUSSION

### 5.1 Effect of shoot bending on changes in endogenous hormones

Flowering in apple trees in the following season depends on the vegetative and reproductive growth at the previous season. It was reported that flower-inhibiting signals are originating in growing shoot tips and seeds, and are subsequently translocated to terminal and axillary buds and spurs (PRANG *et al.*, 1997). Reduction in vegetative growth and/or fruit thinning can enhance the flowering in the following year (WILLIAMS, 1973; DENNNIS, 2003). The orientation in space of the vegetative shoots of apple consist of vertical and horizontal shoots. It has been observed that flowering occur less in the former than the later one (for literature see SANYAL and BANGERTH, 1998). Bending of shoots is therefore a cultural practice that temporarily reduces shoot growth and promote flowering in apple trees, although the physiological mode of action of this method has not been well understood. However, it was reported that changes in endogenous hormones are affected by bending treatments related to FI in pear and apple trees (SANYAL and BANGERTH, 1998; ITO *et al.*, 1999, 2001)

In our experiments, changes in CKs in apical parts after bending treatment showed inconsistent results among different cultivars. In the year 2001 Z/ZR concentrations in vertical shoots of cv. 'Boskoop' and in horizontal shoots of 'Golden Delicious' increased after bending treatment. The tendency could not be confirmed for cv. 'Elstar' and in the seedling experiment. In 2002 it was also found that Z/ZR concentrations in apical parts of cv. Golden Delicious are higher after bending treatments. Changes into the opposite direction were found in cv. Idared. Furthermore, variation of iAde/iAdo concentrations showed no clear tendencies after bending treatment, both in 2001 and 2002. ITO *et al.* (1999) found that zeatin type CKs in lateral buds of pear were higher in vertical bent shoots than vertical control shoots. In contrast to these results, bending treatments in our experiments decreased Z/ZR concentrations in vertical shoots of cv. Boskoop. Furthermore, we found that the Z/ZR concentrations in the vertical shoots were more than in the horizontal shoots. It was indicated that the reduction of Z/ZR concentrations in mechanically stressed subapical shoot tissues might be either directly or indirectly affected by high ethylene accumulation in those tissues (SANYAL and BANGERTH, 1998), whereby the exact relationship between ethylene and CKs is not yet clear. However, some

evidence has been reported that ethylene may decrease CKs in plants (BANKO and BOE, 1975; VAN STADEN *et al.*, 1987).

In recent years, CKs have been related to FI in apple (RAMIREZ, 2000). SKOGERBØ (1992) found that applying zeatin and benzyladenine (BA) directly to the xylem of spurs and elongated shoots of apple could stimulate apical and lateral flowering. CKs coming from the roots act as anti-GAs with respect to FI in buds. Thus, flower formation seems to be related to a certain ratio of GAs and CKs. However, the mediating role of CKs in FI could until now not been proven.

In 2001, IAA concentrations in apical parts of cv. Golden Delicious, Boskoop and Elstar were not different between control and bending treatments until 11 DAT. Interestingly the IAA concentrations were starting to decrease at 16 DAT until 25 DAT (the last sampling date) after bending up and down treatments as compared to control. Reduction of IAA in apical parts of apple seedlings cv. Golden Delicious was also found that started as early as 6 DAT. But the IAA concentrations in leaves behaved in an opposite direction. It was starting to increase also at 6 DAT. This increase in IAA concentration in the leaves of apple seedling might be explained by “IAA-concentration autoinhibition” (BANGERTH *et al.*, 1989).

These results correspond to the experiment in 2002, where there was also a decrease of IAA that started 9 DAT until 11 DAT in cv. Golden Delicious. Interestingly, in cv. Idared the reductions of IAA were found to occur already 3 DAT until 11 DAT (the last sampling date). In many previous bending experiments it was found that mechanically induced stress applied to vigorously growing vertical shoots of apple trees caused a 2 to 2.5 fold decrease in polar auxin transport as compared to vertical control shoots (SANYAL and BANGERTH, 1998). It was suggested that the basipetal IAA-transport from fruits and shoot tips is involved in FI of apple trees (CALLEJAS and BANGERTH, 1997). Furthermore, IAA concentrations and diffusible IAA in shoots of Japanese pear were decreased after bending vertical shoots as compared to control (ITO *et al.*, 1999, 2001). It might therefore be concluded that bending shoots reduces IAA transport and production in buds that are competent to flower and in this way may enhance FI.

In addition also GA concentrations in apical parts of shoot of bearing apple trees of the cvs. Golden Delicious, Boskoop and Elstar started to decline 16 DAT when compared to control shoots. However, 9 days after that the effect of bending on reduction of GAs

seemed to decline, whereas for the bending up treatment still a reduction of GAs for cvs. Boskoop and Elstar when compared to bending down and control was noticed. Lower concentrations of GAs after shoot bending were also found in seedling experiments. Here GA concentrations in apical part of shoots and leaves were reduced from 2 DAT until 12 DAT. In 2002, these tendencies in GA concentrations in apical part of shoots after bending were, however, different. Reduction of GAs was found at the beginning of the sampling period, 1-3 DAT, in cv. Golden Delicious as compared to control, while there were no clear tendency observed in cv. Idared. ITO *et al.* (1999, 2001) also found that GA concentrations in shoots of Japanese pear were decreased after bending vertical shoots as compared to control. Another vegetative growth restriction, e.g. girdling of branches, also result in a reduction of GAs in the xylem sap of peach (CUTTING and LYNE, 1993).

GAs were reported as inhibitors of flower induction in many fruit trees (DAVENPORT and NUNEZ-ELISEA, 1997). CALLEJAS (1999) indicated that application of GAs reduces the number of flowers in apple trees the following year. Furthermore, he found that GAs enhanced IAA diffusion from fruits and shoot tips. For this reason it has not been clarified whether GAs act by themselves as flower inhibiting signal or by stimulating IAA as a secondary flower inhibiting signal.

In the above experiments shoot bending reduced the concentrations of GAs, IAA and diffusible IAA in terminal parts of shoots earlier or later and at different degree depending on vegetative growth, which is related to cultivars, which is stronger for 'Boskoop' and 'Elstar' than for 'Golden Delicious' (SILBEREISEN *et al.*, 1996). ABA concentrations showed inconsistent tendencies after bending treatments. ABA is regarded as a hormonal stress signal, produced in roots or mature leaves (HARTUNG *et al.*, 2002). It was considered as a possible hormone stimulating FI in apple (LUCKWILL, 1974). ITO *et al.* (1999) found that bending shoots increased ABA concentrations in lateral buds of Japanese pear. However, such a role of ABA in FI has been found only in a few cases (BUBÁN, 2003). HOAD and Hughes-Games (1983) reported that no correlation could be found between the amount of diffusible ABA and the return bloom of apple, pear and plum.

Shoot bending alters the concentrations of plant hormones in different ways depending on apple tree cultivars etc. However, most results showed a reduction of GA and IAA concentrations when compared to control. It is known that the GAs, GA<sub>3</sub> and GA<sub>4/7</sub>, inhibit flower induction in apple trees. IAA export out of shoot tips and seeds of fruits

were also reported to presumably inhibit FI of apple in the following year. Furthermore, GA application increased the amount of IAA exported from shoot tips (CALLEJAS and BANGERTH, 1997). It might therefore be concluded that high concentrations of GAs and IAA are undesirable conditions for FI of apple trees and could, as shown above, been positively modified by bending thus increasing FI (Figure 5.1).

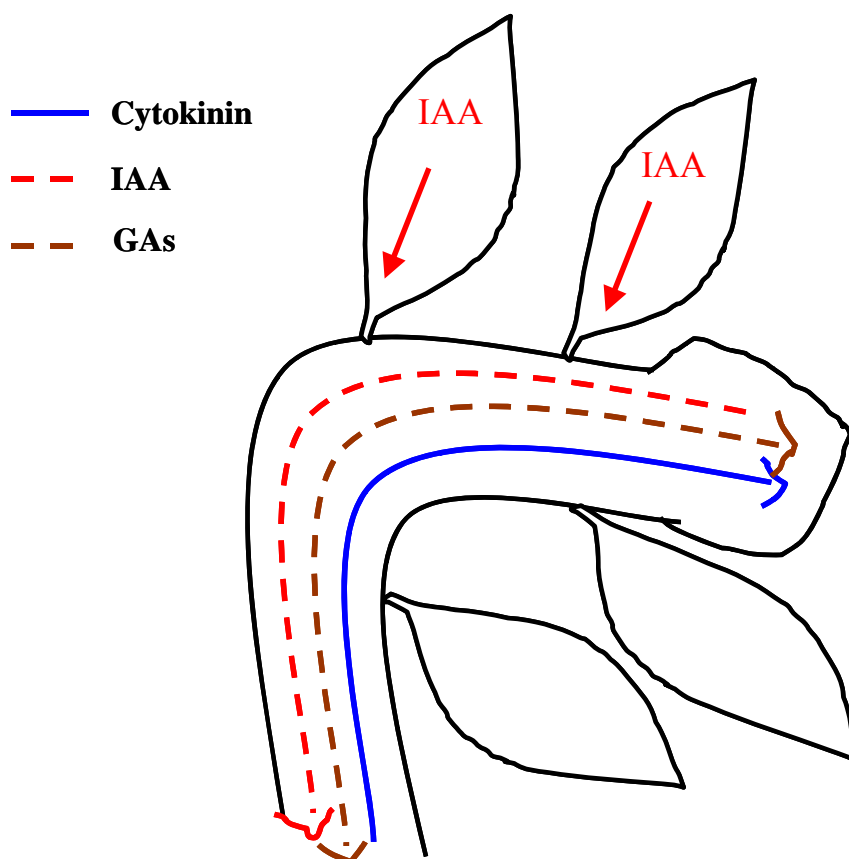


Figure 5.1 Direction of hormone transport related to flower induction in horizontally bended apple shoots of cv. 'Golden Delicious'. Solid arrows presumably indicate flowering inducing, dotted arrows flowering reducing hormone concentrations. The role of the IAA transport out of leaves is not yet clear but may well have a negative function by inhibiting IAA transport out of terminal bud

## 5.2 Effect of spraying Alar and Ethrel on changes in endogenous hormones

Reduction of vegetative growth by spraying particular growth retardants has been demonstrated to promote FI in apple and other fruit trees (TROMP, 1972, LUCKWILL and SILVA, 1979; MACLAUGHLIN and GREENE, 1984; ITO, *et al.*, 2001, ELFVING,

*et al.*, 2003). CALLEJAS (1999) reported that Alar + Cerone ( tradename of a particular ethylene formulation) and TIBA + Cerone treatments enhanced flowering in apple trees in the following year. However, there have been only few investigations of any relationship to changes in endogenous hormone conditions. In the above experiments, during the years 2001, 2002 and 2003, a combination of 1000 ppm Alar and 250 ppm Ethrel was sprayed on apple trees cv. ‘Golden Delicious’, ‘Elstar’, ‘Boskoop’ and ‘Idared’. The results revealed that this spraying treatment increased the concentrations of CKs, especially in growing shoots, as compared to controls, except for cv. ‘Boskoop’, which showed the opposite result. Furthermore, increase in CKs in wood and bark were found at 2 and 3 days after spraying.

RAMIREZ and HOAD (1981) reported that CK levels in buds were higher in ‘Cox’s Orange Pippin’ after Alar treatment. It was also reported that foliar application of maleic hydrazide (MH) at 2600 ppm increased Z/ZR and iAde levels but decreased iAdo in lateral buds of Japanese pear (ITO *et al.*, 2001). Paclobutrazol (PBZ), a GA biosynthesis inhibitor that can promote flowering in mango trees, also increased CK concentrations in apical shoots, wood and bark of mango trees (NAPHROM, 2004).

In 2001, changes in GA concentrations of growing shoots showed no clear tendency in cvs. ‘Golden Delicious’ and ‘Boskoop’ after the spraying Alar plus Ethrel, whereas the GA concentrations in non-growing shoots were decreased in both cultivars as compared to control. However, in 2002 and 2003 the GA concentrations in growing shoots of cv. ‘Golden Delicious’ and ‘Elstar’ also declined after spraying Alar plus Ethrel when compared to untreated trees, whereas there was no clear tendency in non-growing shoots. Furthermore, a significant reduction of GA concentrations in wood and bark could also be detected after spraying Alar plus Ethrel. However the responsiveness of growing and non-growing shoots to growth retardants in terms of hormonal changes is still inconsistent and little understood. Perhaps it depends on the difference in the strength of the vegetative growth in each cultivar and on the different climatic conditions. Reductions of GAs under the influence of growth retardants treatment have been frequently reported. HOAD and MONSELISE (1976) demonstrated that Alar reduced gibberellin-like activity in shoot tip extracts of M26 apple rootstock as compared with unsprayed trees. RYUGO and SANSAVINI (1972) applied Alar to young sweet cherry trees and also found that extractable GAs and diffusible GAs from apices of upright shoots were decreased. It was reported that application of exogenous GAs caused inhibition of FI in many fruit trees.

BERTELSEN and TUSTIN (2002) among many others reported that GAs inhibit flower bud formation in the cv. 'Pacific Rose' apple.

IAA concentrations in growing shoots were decreased after spraying Alar and Ethrel as compared to untreated trees, whereas in non-growing shoots a reduction of IAA concentrations could not be detected. Growing shoots are major sites of IAA biosynthesis. Therefore, their responsiveness to plant growth retardants in inhibiting IAA biosynthesis was more pronounced as compared to non-growing shoots, which produce less IAA. The results showed the same tendencies in all cultivars and during 3 years of experiments. Furthermore, IAA export out of shoots was also considerably reduced after spraying treatments as compared to control. In other previous experiments, CALLEJAS (1999) indicated that IAA export out of terminal shoots was decreased at 23, 27 and 30 days after full bloom after spraying with Alar + Cerone and TIBA + Cerone. WILLIAMS (1973) mentioned that TIBA, Alar and Ethrel are all effective in repressing IAA and GA production and distribution in actively growing shoots of apple trees. Growth retardants might decrease the export of flower inhibiting signals, such as GAs and/or IAA, from growing shoots to terminal buds spurs, where FI will take place. There are some evidences that suggest a high correlation between FI and polar IAA export (BANGERTH, 1993; SANYAL and BANGERTH, 1998).

In the year 2001, spraying Alar and Ethrel seemed to decrease ABA in growing and non-growing shoots of cv. 'Golden Delicious' and in non-growing shoots of cv. 'Boskoop' about 5 DAT until 10 DAT. But there were no clear tendencies in 2002. HOAD and MONSELISE (1976) mentioned that growth retardants on changes in endogenous ABA of fruit trees have not been so far investigated. However, reduction of ABA levels after triazole treatments has been reported for apple, rape and soybean (WANG *et al.*, 1985; HAUSER *et al.*, 1990; GROSSMANN, 1990).

In the above experiments, both shoot bending and chemical treatments, showed common signs of reductions of GAs and IAA in the shoots, which corresponds with some other previous experiments. The role of ABA in FI of fruit trees is however still unclear. It might be concluded that FI in apple trees requires low concentrations of GAs and/or IAA and high concentrations of CKs. Optimum balances of these phytohormones to control flowering needs further experiments to be proven.

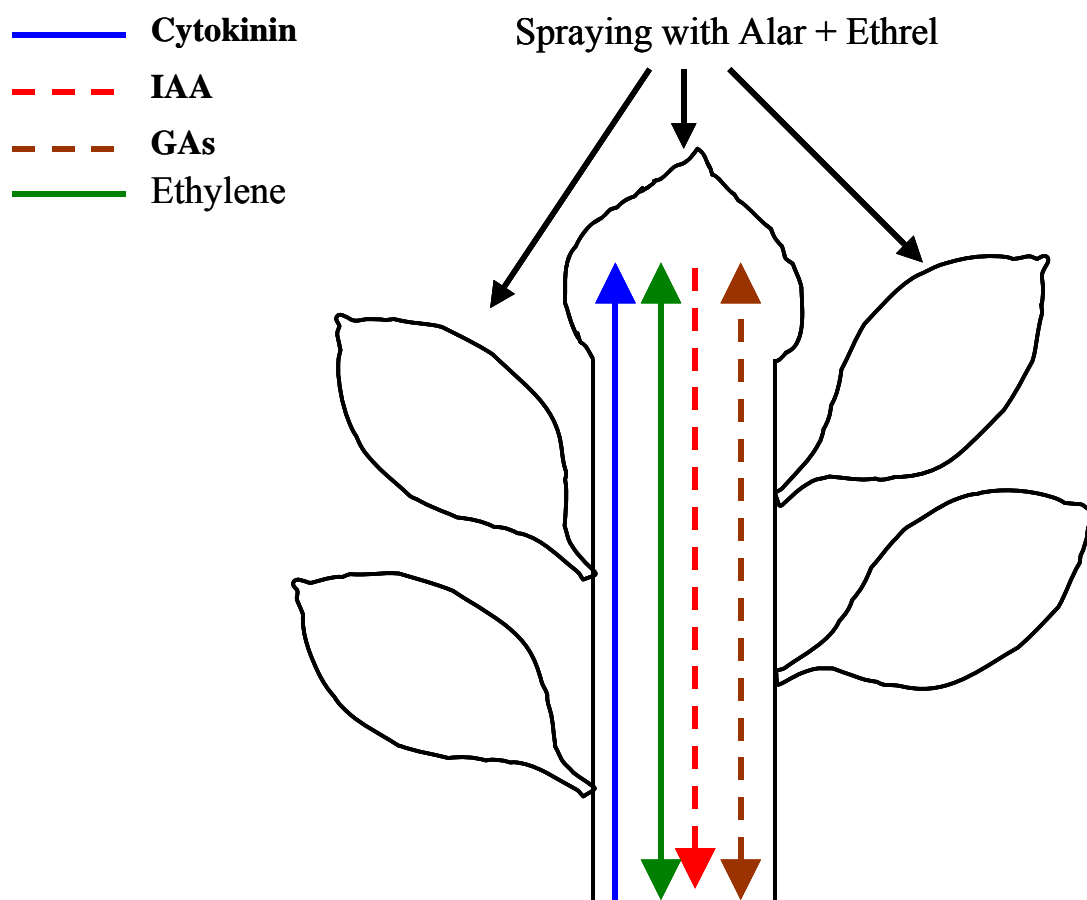


Figure 5.2 Changes in endogenous hormones in the apical part of shoot of apple cv. 'Golden Delicious' after spraying with Alar plus Ethrel. Solid arrows indicate flowering inducing concentration. Dotted arrows indicate reduced concentration.

### 5.3 Carbohydrate contents

It was reported that carbohydrates might limit the formation of flowers in fruit trees during or following a heavy cropping season (SACHS, 1977, GOLDSCMIDT and GOLOMB, 1982, WHILEY et al., 1989). The production of a high yield presumably depletes carbohydrate reserves and thereby may inhibit flower bud initiation (CHILDERS, 1961).

Sucrose levels reaching the apical bud had been a key factor to theories of FI (SACHS, 1977). It has been believed that the increment of flower bud formation induced by applications such as growth retardants, ringing, root pruning, and fruit thinning all might be somehow related to increase in carbohydrates at the site of flower formation (LUCKWILL, 1970, JACKSON and SWEET, 1972). But until today there are no direct evidences demonstrating that carbohydrates control FI of fruit trees. DAVENPORT and NUNEZ-EILISEA (1997) indicated that flower initiation might occur when a critical threshold level of carbohydrate is reached in buds together with a putative floral stimulus. In the above experiment it seemed to be no positive correlation between changes in carbohydrate contents (reducing sugar, sucrose and starch) caused by spraying treatments with the exception of reducing sugar concentrations, which were increased in growing and non-growing shoots of cv. Elstar after spraying Alar and Ethrel.

These findings are contradictory to previous experiments. FILIPOVICH and ROWE (1977) e.g. found that spraying young apple trees with 2000 ppm of Alar resulted in a significant increase in starch contents in the whole plant, bark, wood and roots. Furthermore, it was observed that the translocation of  $^{14}\text{C}$ -labelled assimilates to the shoot tips of apple seedlings was reduced within 10-14 days after the application of 400 ppm of SADH (MONSELISE and LUCKWILL, 1974). ULGER *et al.* (2004) found that differences in concentrations of any sugar, except fructose, were not significant in on and off years of olive trees. It was also reported that carbohydrate concentrations in the bud of Japanese pear were not significantly different between regular (Chojuro) and irregular bearing (Kosui) cultivars (ITO *et al.*, 2002). They suggested instead that sugar metabolizing enzyme activities were involved in bud development. Higher activities of sugar catabolizing enzymes may enhance the capacity of buds to attract assimilates, thereby accelerating bud growth, whereas the role of sugar concentrations in bud morphogenesis remains unclear. There was also no evidence that high starch levels promote FI in mango trees (WHILEY *et al.*, 1989). CHADHA and PAL (1986) concluded that carbohydrate reserves play an important role in flower bud initiation of mango trees, though they are not the primary factor.



## 6 CONCLUSION AND OUTLOOK

According to literature shoot bending and spraying the growth regulators Alar plus Ethrel, affects flower induction treatments possibly mediated by changes in endogenous hormones in various tissues of apple trees. The above demonstrated changes in hormone concentrations partly support these assumptions but were little consistent from year to year. These inconsistent results can possibly be explained by the observed varying climatic conditions, in particular the considerable differences in temperature and rainfall. Beside these climatic reasons the results also revealed that the cv. 'Golden Delicious' with more vigorously growing shoots behaved more responsive to the applied treatments than the other cvs. and the non-growing bourse shoots as far as changes in endogenous hormones are concerned. There were no obvious positive correlations observed between changes in carbohydrate contents and bending or spraying with Alar plus Ethrel. Carbohydrate reserves may be essential for flower induction, but they are not floral signals. Particular changes in phytohormones are obviously a prerequisite for flower induction but is likely that a particular balance of them is necessary for flower induction.

It might be concluded that flower induction in apple trees needs higher concentrations of cytokinin and ethylene and lower concentrations of IAA and GAs. Furthermore IAA in shoot exudates was decreased after spraying with Alar plus Ethrel. These phenomena are similar to flower induction in mango and longan trees. Reduction of basipetal IAA export from shoot tips may release cytokinin production in roots which than is transported via xylem acropetally in responsive buds. On the other hand free cytokinin may be released from conjugated cytokinins, which accumulated in apical buds and the subtending stem. In further experiments these conjugated cytokinins, and possibly conjugated IAA and GAs as well should be investigated. Other possibilities to increase cytokinin and ethylene or decrease IAA and GA concentrations should be discovered to promote flowering.

## 7 SUMMARY

Apples are cultivated commercially throughout the temperate zone. A regular production however does not seem possible because of irregular yields from year to year. Main causes for this are the so called "alternate bearing" behavior which is the result of profuse flowering in one year but few or no flowers in the following year. It is reported that too vigorously growing shoots are part of the reasons for alternate bearing in apple trees. Applications of chemicals or conventional cultural practices, such as bending shoots have been widely used to restrict shoot growth and promote flower induction (FI). However, the physiological mode of action of these methods in FI is still unknown. Phytohormones are thought to be involved in the process of flower induction. In the above experiments, we investigated changes in endogenous hormones, starch and sugar contents after bending upright shoots into a horizontal position and spraying apple trees with the growth regulators Alar plus Ethrel to improve FI. The experiments were carried out during the years 2001 to 2003 at the Experiment Station, of the University of Hohenheim, Germany, whereby the apple cvs. 'Golden Delicious', 'Boskoop', 'Elstar' and 'Idared' were used. The apical part of growing shoots and non-growing bourse shoots, beside bark, wood and shoot diffusates were collected. Plant samples were frozen immediately in liquid nitrogen and freeze dried. Phosphate buffer 0.1M, pH 6.2 was used for collecting auxin in the shoot diffusates. All samples were stored at  $-20^{\circ}\text{C}$  until extraction and purified, identified and quantified by Radio Immuno Assay (RIA).

The results revealed, in general, that shoot bending and spraying with Alar plus Ethrel changed the endogenous hormone concentrations in the apical part of shoots, as well as in wood, bark and shoot exudates of apple trees. The 'Golden Delicious' cultivar and vigorously growing shoots showed clearer tendencies of hormonal changes than the other cvs. and non-growing bourse shoots. Cytokinin concentrations in the apical part of shoots, and in wood and bark increased after both treatments. Contrary to that, GAs and IAA concentrations in the apical part of shoots and in shoot exudates showed the opposite results. Both treatments had no effect on the concentration of ABA. Ethylene production in shoot tips was considerably stimulated by the combined treatment of Ethrel plus Alar probably due to Ethrel being a "synthetic precursor" of ethylene. Considerable variation existed in the mentioned hormonal changes in respect to the year of examination and the cv. under investigation. Time of treatments and in particular climatic conditions were

probably the most influential variables. In spite of all this and on the basis of the above results the conclusion can be drawn that higher concentrations of cytokinins and lower concentrations of gibberellins and auxin are favorable for FI.

Spraying with Alar plus Ethrel and bending of shoots seemed to decrease the reducing-sugars, as well as sucrose and starch concentrations in growing shoots and their leaves. In non-growing shoots, spraying seemed to reduce starch but to increase reducing-sugars and sucrose concentrations. A correlation between changes in carbohydrate contents (reducing sugar, sucrose and starch) caused by the spraying treatments and FI does not seem to exist. All the observed changes in the carbohydrate concentrations caused by spraying treatments were not particularly impressive and did not really support the often published claim that the effect of spraying growth regulators, bending shoots or other cultural practices may mediate their stimulatory effect on FI via a change in carbohydrates. In contrast to that the above observed experimental results rather suggest that hormones are more effectively involved in the flower induction process of fruit trees.

## 8. ZUSAMMENFASSUNG

Kommerzieller Apfelanbau findet im gesamten gemäßigten Klimabereich statt. Die Produktion ist jedoch über die Jahre hinweg nicht konstant. In vielen Anbaugebieten schwanken daher die Produktionszahlen erheblich. Hauptgrund hierfür ist die Alternanz, was soviel bedeutet wie, dass die Apfelbäume in einem Jahr sehr stark, im Folgejahr hingegen kaum oder überhaupt nicht blühen und fruchten. All zu kräftiges Triebwachstum wird als eine der möglichen Ursachen der Alternanz von Apfelbäumen genannt. Es ist weithin bekannt, daß die Anwendung gewisser synthetischer Wachstumsregulatoren oder bestimmte Anbaumaßnahmen dazu geeignet sind das Triebwachstum zu begrenzen und die Blüteninduktion (BI) von Apfelbäumen zu fördern. Der im Hinblick auf die Blüteninduktion physiologisch wirksame Mechanismus dieser Verfahren ist jedoch immer noch weitgehend unbekannt. Intensiv wird eine Beteiligung von Phytohormonen an diesem Prozeß der BI diskutiert. In den vorliegenden Experiment untersuchten wir neben den Veränderungen der endogenen Hormonkonzentrationen auch diejenigen des Stärke- und Zuckergehalts als Folge des Herunterbiegens von Trieben, sowie des Spritzens von synthetischen Wachstumsregulatoren mit dem Ziel der Verbesserung der BI. Die Versuche wurden in den Jahren 2001 bis 2003 an der Versuchstation der Universität Hohenheim durchgeführt, wobei die Sorten "Golden Delicious", "Boskoop", "Elstar" und "Idared" aufgrund ihres unterschiedlichen Alternanzverhaltens als Versuchsobjekte ausgewählt wurden. Dazu wurden die Triebspitzen wachsender Langtriebe und nicht-wachsender Fruchtspieße (bourse shoots), sowie Rinde, Holz und Sprossspitzendiffusate gesammelt. Das Pflanzenmaterial wurde unmittelbar in flüssigem Stickstoff schockgefroren und gefriergetrocknet. Zur Gewinnung der Auxin-Sprossspitzendiffusate wurde 0,1M Phosphatpuffer, pH 6,2 verwendet. Alle Proben wurden bis zu ihrer Analyse bei -20°C gelagert. Das Pflanzenmaterial wurde über Nacht in 80 prozentigem kaltem Methanol extrahiert und über eine Kombination aus PVP, DEAE-Sephadex A-25 Säulen sowie Sep-Pak C<sub>18</sub> Kartuschen aufgereinigt. Der Gehalt an den endogenen Hormonen Cytokininen (CKs), Gibberellinen (GA<sub>1,3,20</sub>= GAs), Abszisinsäure (ABS) und Auxin (IES) wurde mittels Radioimmunoassay (RIA), Ethylen mittels GC qualitativ und quantitativ bestimmt.

Generell wiesen die Ergebnisse auf veränderte Hormonkonzentrationen in Triebspitzen, Rinde, Holz und Triebexsudaten infolge des Biegens, bzw. Spritzens mit Alar und Ethrel hin. Wachsende Triebe im allgemeinen und die Sorte "Golden Delicious" im besonderen zeigten deutlichere Tendenzen als das übrige Pflanzenmaterial. Hier zeigte sich, dass die

Cytokinin-Konzentrationen in den Triebspitzen, Holz und Rinde nach beiden Behandlungsmethoden deutlich anstiegen. Im Gegensatz zu den Cytokinin-Konzentrationen zeigten die GAs- sowie IES Konzentrationen in Triebspitzen und Triebexsudaten nach Biegen und Wachstumsregulator Applikation rückläufige Tendenzen. Infolge der Spritzungen konnte auch ein Anstieg der Ethylen-Konzentrationen gemessen werden, vermutlich weil Ethrel eine synthetische Vorstufe von Ethylen darstellt. Ein Einfluß der Behandlungen hinsichtlich der ABS-Gehalte war nichterkennbar. Diese Ergebnisse stehen mit einigen Ergebnissen früherer Experimenten im Einklang.

Für das über die 3 Jahre und 4 Sorten beobachtete nicht immer konstante Verhalten bezüglich der Hormon und Kohlenhydrat Veränderungen kann man wahrscheinlich das Alter der Bäume, den Zeitpunkt der Behandlungen sowie unterschiedliche klimatische Bedingungen verantwortlich machen. Aus den vorliegenden Ergebnissen könnte die Schlussfolgerung gezogen werden, daß die BI von Obstbäumen hohe CKs-Konzentrationen und niedrige GAs und IES-Gehalte voraussetzt.

Die Spritzung von Alar und Ethrel schien zu einer Verringerung der reduzierenden Zucker, Saccharose und Stärke in wachsenden Apfel-Trieben und ihren Blättern zu führen. In nicht wachsenden "bourse-Trieben" hingegen zeigten sich zwar die Stärkegehalte aufgrund der Spritzung verringert, die Konzentrationen an reduzierenden Zuckern und Saccharose wurden jedoch durch die Behandlung erhöht. Im Ganzen gesehen scheint jedoch keine positive Korrelation zwischen veränderten Kohlehydratgehalten und den Spritzbehandlungen zu existieren. Bislang wurde häufig angenommen, dass der Anstieg der Blütenknospenbildung, z.B. wie er nach Anwendung von Wachstumsretardanzien, oder agronomischer Maßnahmen, wie Ringelung, Wurzelschnitt und Fruchtausdünnung zu beobachten, in irgendeiner Weise mit einem Ansteigen der Kohlehydrate an den Orten der Blütenbildung in Verbindung stehe. Bislang gibt es dazu jedoch keinerlei direkte Beweise. Diese Befunde lassen u.U. den Schluss zu, dass den Kohlehydrat-Reserven eine gewisse Bedeutung bei der Induktion von Blütenknospen zukommt, dass sie dabei jedoch nicht die Schlüsselrolle spielen.

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